CE LIBRARY

MAY 23 1929

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

VOL. LVI MARCH, 1929 No. 3

CONTENTS

THORNER, MELVIN W. *Recovery of the Heart Beat of Fundulus Embryos After Stoppage by Potassium Chloride.* 157

REICHLE, HERBERT S. *The Diagnosis of the Type of Twinning*..... 164

WHEDON, ARTHUR D. *Muscular Reorganization in the Odonata During Metamorphosis*..... 177

SUMWALT, MARGARET. *Potential Differences Across the Chorion of the Fundulus Egg*..... 193

GEROULD, JOHN H. *History of the Discovery of Periodic Reversal of Heart-Beat in Insects*..... 215

LAMBERT, W. V., AND CURTIS, V. *Further Studies on the Sex Ratio in the Chicken.* 226

PUBLISHED MONTHLY BY THE
MARINE BIOLOGICAL LABORATORY

PRINTED AND ISSUED BY
LANCASTER PRESS, INC.
LANCASTER, PA.

AGENT FOR GREAT BRITAIN
WHELDON & WESLEY, LIMITED
2, 3 and 4 Arthur Street, New Oxford Street, London, W. C. 2

Single Numbers, $1.00. Per Volume (6 numbers), $4.50

Entered October 10, 1902, at Lancaster, Pa., as second-class matter under Act of Congress of July 16, 1894

Editorial Staff

GARY N. CALKINS—*Columbia University.*
E. G. CONKLIN—*Princeton University.*
M. H. JACOBS—*University of Pennsylvania.*
FRANK R. LILLIE—*University of Chicago.*
GEORGE T. MOORE—*The Missouri Botanic Garden.*
T. H. MORGAN—*Columbia University.*
W. M. WHEELER—*Harvard University.*
E. B. WILSON—*Columbia University.*

Managing Editor

C. R. MOORE—*The University of Chicago.*

All communications and manuscripts should be sent to the Managing Editor, the University of Chicago. Subscriptions and other matter should be addressed to the Biological Bulletin, Prince and Lemon Streets, Lancaster, Pa.

Vol. LVI *March, 1929* *No. 3*

BIOLOGICAL BULLETIN

RECOVERY OF THE HEART BEAT OF *FUNDULUS* EMBRYOS AFTER STOPPAGE BY POTASSIUM CHLORIDE.

MELVIN W. THORNER,

ZOÖLOGY LABORATORY, UNIVERSITY OF PENNSYLVANIA AND THE BIOLOGICAL LABORATORY, COLD SPRING HARBOR.

INTRODUCTION.

Loeb and Cattell (1) found that when the embryonic hearts of fertilized *Fundulus* eggs have begun to pulsate, they may be completely stopped by placing the eggs in a solution of KCl. They found further that when eggs so treated were placed again in sea water, the hearts resumed beating. These authors also pointed out that "embryos whose hearts had stopped beating under the influence of a sufficient dose of KCl did not begin to beat when put into distilled water." However, in another part of the same paper a table is given in which it is shown that three of thirty of the hearts stopped by KCl recovered in distilled water after a short period. The experiment was discontinued at the end of this time.

Although Loeb and Cattell noted these facts, they failed to extend their investigations in a quantitative manner. This paper endeavors to determine the quantitative relationships of the various factors concerned in effecting recovery of the heart-beat after stoppage by KCl. This recovery was studied in sea water, distilled water and in various salt solutions.

MATERIAL.

The material used was the developing egg of *Fundulus heteroclitus*, collected at the Biological Laboratory, Cold Spring Harbor. The eggs were "stripped" from the females within an hour of

the time the fish were taken from the ocean. The eggs were then immediately fertilized by a sperm suspension milked from the males. The egg stock thus prepared was kept at room temperature (22–24° C.) in sea water changed daily.

At the end of the fourth day after fertilization the heart normally begins to pulsate. All experiments were carried out at room temperature (22–24° C.). The heart was considered as "stopped" when all three chambers had ceased pulsating.

Experiments.

(*a*) *Recovery in Sea Water.*

The first set of experiments represents an attempt to determine the relationship between the time of immersion in KCl and the time of recovery in sea water. Also different concentrations of KCl were tried to see if the relationship varied for different strengths. For this purpose lots of from 40–75 eggs were chosen for each experiment. The heart of each embryo was observed to be in good physiological condition. Each lot was placed in a

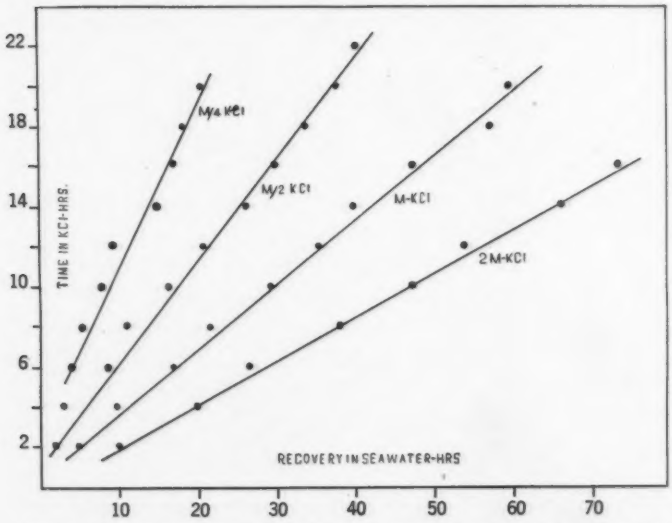


Fig. 1. Shows relation between period of recovery in sea water and period of immersion in different strengths of potassium chloride for hearts of Fundulus embryos.

KCl solution, the strength of which varied from 0.25 M to 2.00 M, for a period varying from 2 to 22 hours. The eggs were then rinsed in distilled water and placed again in sea water and observed at intervals and the time of recovery (*i.e.*, recovery of all three chambers) for each egg noted and the average time computed. By rinsing the eggs in two changes of distilled water each time they were to be placed in a new solution, the possible salt antagonism at the surface of the egg was reduced. At the same time the immersion in the distilled water was too short to cause permeability changes in the membrane. Fig. 1 shows results of such experiments.

From Fig. 1 it may be seen that the time of recovery is directly proportional to the duration of immersion in KCl solution—all other factors remaining constant. This relationship holds within the limits of 0.25 M and 2.00 M.

The next experiments to be tried were an endeavor to determine the relationship between the strength of KCl used and the period of recovery in sea water. Again lots of 40–75 eggs were selected. Each lot was placed in a KCl solution of from

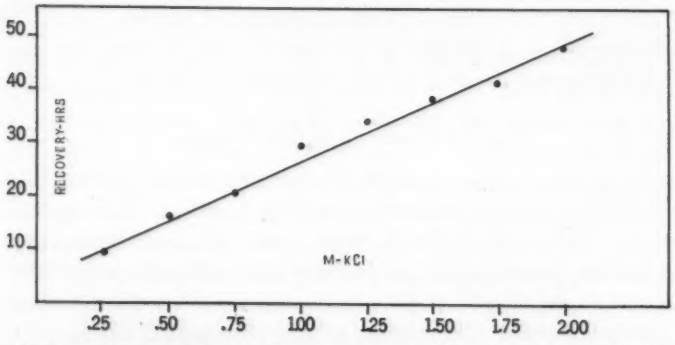


FIG. 2. Shows relationship between concentration of potassium chloride and period of recovery in sea water for hearts of Fundulus embryos after immersion in KCl for ten hours.

0.25 M to 2.00 M for ten hours. The periods of recovery were noted and averaged as before. Fig. 2 shows the results of this group of experiments.

From Fig. 2 it is evident that the duration of the period of re-

covery is directly proportional to the concentration of KCl used.

It was then decided to determine the relation of strength of KCl to the time required for complete heart cessation at the various strengths (Fig. 3).

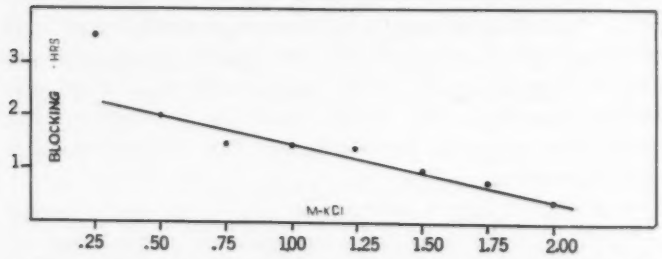


FIG. 3. Shows relationship between molar strength of potassium chloride and the time required for complete heart cessation in Fundulus embryos.

Figure 3 suggests the possibility of a direct proportionality. If the assumption of Loeb is correct, *i.e.*, that the same amount of KCl is needed each time to block completely the heart-beat and if the rate of entry of KCl is directly proportional to the concentration of KCl, the relation between concentration and time should be represented by a straight line. This is approximately true and hence it is probable that Loeb's assumption is correct.

(*b*) *Recovery in Distilled Water.*

Lots of 200 eggs each were selected one by one, washed free of adhering salts and placed for varying lengths of time in M/2 KCl. They were washed in several rinsings of distilled water. Then they were placed in distilled water changed daily. The period of recovery in distilled water was noted for the individual eggs at intervals. The average of each lot was then calculated.

From Fig. 4 it may be seen that, contrary to the opinion of Loeb, recovery of an appreciable number of eggs is effected in distilled water. Instead of merely non-recovery, coagulation of the embryo was used as a criterion of death and each egg was carried through to either coagulation or recovery. It was found that mortality increases with the length of immersion in KCl. The extreme variation from the mode also increases with the length of immersion.

In an attempt to confirm Loeb's statement (1) that "KCl cannot diffuse out of *Fundulus'* egg in distilled water" and assuming that it does diffuse in instead of remaining at the vitelline membrane, we endeavored to discover why the heart-beat recov-

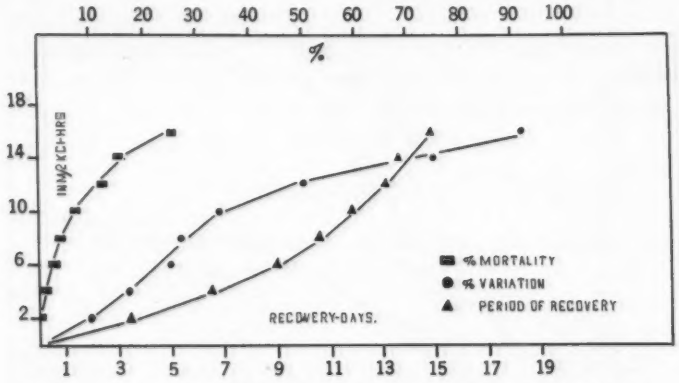


FIG. 4. Shows relationship between time for recovery in distilled water and length of exposure to M/2 KCl for hearts of Fundulus embryos. The percentage mortality and percentage variation for same eggs are also indicated.

ered in distilled water. Three lots of 200 eggs each were selected, washed and placed in M/2 KCl for six, eight and twelve hours, respectively. Then each lot of eggs was washed and put into 40 mls. of distilled water (in a 200 × 25 mm. test tube) changed daily. The daily *increase* in electrical conductivity was followed. (Before each conductivity measurement the water was boiled momentarily to free it from CO_2). Limited time made necessary the omission of a quantitative chemical analysis of the K content but conductivity measurements furnish a possible indication of the K extruded.

The graph of Fig. 5 shows that some electrolytic substance was extruded through or at least given off from the membranes of the egg. The fact that the hearts began to beat again leads one to suppose that it is at least partly KCl. With one exception there was found to be a daily increase in the rate of extrusion. The rate increased most just before recovery of the hearts began to be manifest. The amount extruded was dependent on, but not proportional to the length of immersion in M/2 KCl.

It is readily apparent that the mechanism of recovery in sea water and in distilled water is fundamentally different. That in sea water is affected by concentration and duration of immersion

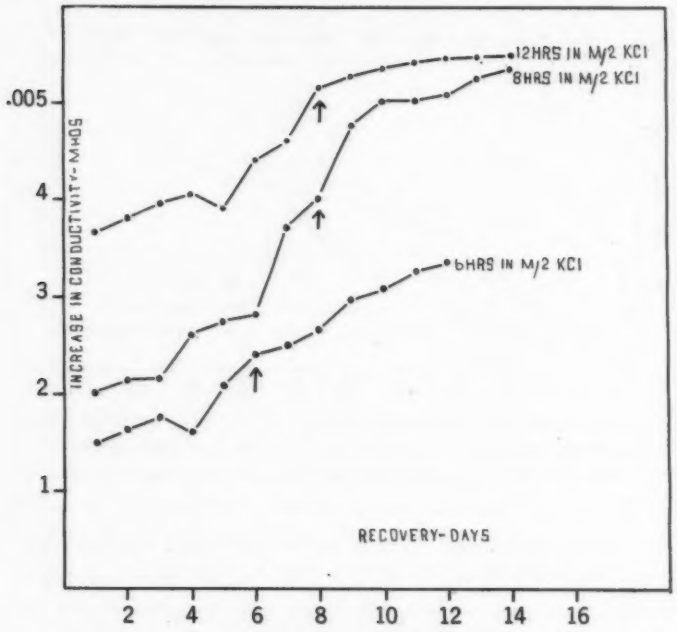


FIG. 5. Shows increase in electrical conductivity of distilled water in which eggs, immersed for different periods in M/2 KCl are placed. Arrows indicate time heart resumed beating.

in KCl in direct proportionality. The recovery in distilled water is more complicated.

Loeb (1) noted recovery with various cations and anions. The cations here tried effect a momentary recovery. This is probably not due to salt antagonism but to a direct overstimulation resulting in death of the temporarily aroused hearts.

SUMMARY.

1. The heart-beat of eggs blocked completely by KCl recover in both sea and distilled water although the two recovery processes are dissimilar.

2. In sea water the duration of the period of recovery is directly proportional to the concentration of KCl and to the time of immersion in KCl. The same amount of KCl is required to block completely the heart-beat.

3. The recovery of eggs in distilled water was studied with the aid of electrical conductivity.

I am deeply indebted to Dr. J. H. Bodine for suggestion of the problem and for his valuable assistance and advice. I am also indebted to Dr. R. G. Harris, Director of the Biological Laboratory, for making research facilities available to me.

LITERATURE CITED.

1. **Loeb and Cattell.**

'15 The Influence of Electrolytes upon the Diffusion of Potassium out of the Cell and into the Cell. J. Biol. Chem., 23, p. 41.

THE DIAGNOSIS OF THE TYPE OF TWINNING.

I. DERMATOGLYPHICS.

HERBERT S. REICHLE, M.D.[1]

In the first systematic study of monoovular twins Galton (1) in 1883 demonstrated its unique value in genetics. Little further advance was made until recent years. The emphasis placed upon dramatic but unusual cases of identity had given a romantic and burlesque character to this subject and had inhibited methodical research. Wilder's (2) studies of palm prints and still more Siemen's (3) ingenious diagnostic scheme have opened this field anew to scientific inquiry.

The interest which the monoovular twin—abbreviated in this article to M. T.—holds for biologists lies in the identity of their germ plasm. The two individuals constitute a natural clone—an experiment out of nature's laboratory by which we might hope to separate the influences of inheritance from those of environment. By the latter term we signify all those extrinsic factors which act upon the cell and its successive forms from the moment of conception. Complications such as idiokinesis, unequal equatorial division and extraordinary parakinetic influences in utero may render the interpretation difficult. A few writers (4) regard these phenomena as not uncommon. They, therefore, question the value of the method. It is impracticable for us to discuss these objections in the present paper. We realize that the entire structure of our investigation rests upon the assumption that these doubts are unjustified. We believe that a vast weight of authority (5) accepts the truly equatorial division of the chromosones and denies the somatic segregation of characteristics except in special instances. Moreover, M. T. are universally regarded as the product of one sperm and one ovum. Without the first two assumptions in particular the Mendelian theory is an incongruity.

The chief difficulty lies not in these theoretical objections but

[1] From The Babies' and Children's Hospital of Cleveland and the Department of Pediatrics, Western Reserve University.

in the practical insecurity of the diagnosis of monoovularity. In a future paper we shall discuss the different methods that have been proposed. We reserve for this publication the one first suggested by Wilder—the identity of palm and sole prints.

The first part of our work was an investigation of the dermatoglyphics of double monsters (cosmobii), the only twins about whose monoovularity there can be no doubt. We then examined forty pairs of non-conjoined twins and attempted to correlate their palm prints with sex likeness and the data obtained by Siemen's method of twin study. We have selected from the very extensive literature on twins and twin diagnosis only such papers which bear directly upon our main argument, which is, that dermatoglyphics is not a reliable method of determining whether twins are monoovular or diovular.

The early history of the work on skin patterns can be found in the publications of Wilder (2) and Schlaginhaufen (6). The latter gives a complete discussion of the anatomy, embryology and comparative anatomy. In Mrs. Wilder's article (Inez Whipple) (7) the last subject is exhaustively treated. The epidermal ridges, which we shall discuss, have nothing in common with the lines of the palmist. The latter are mere folds of the skin that follow the direction of joint movement. The lines with which dermatoglyphics is concerned appear on the surface of the skin as ridges and intrude as thin papillæ into the corium. Through these papillæ the sweat gland ducts pass to empty at the summit of the ridge; Blaschko, therefore, called them "Druesenleisten." Between each pair of Druesenleisten there is another shorter papilla which corresponds to the epidermal furrow of the upper skin layers; these are the folds of Blaschko. According to Schlagenhaufen (6) the epidermal ridges appear in the fourth month of prenatal life whereas the Druesenleisten are not definitely defined before the fifth and the folds of Blaschko not before the sixth months. Schultz (8), however, found no trace of epidermal ridges either on soles, palms or tails of Guiana Howling monkey foetuses which had reached the second stage of their development —which is perhaps equivalent to the twentieth week of human ontogeny. From both observations we can conclude that the ridges

and the subsequent patterns are relatively late structures—an important fact in any consideration of their genetic value.

There seems to be no doubt that the patterns found on palms, soles and finger tips are the result of the surge of lines about the interdigital and apical pads of the lower animals. Traces of these pads can still be seen on some adults and they are well developed in the human foetus. As these pads degenerated or became modified, the patterns have undergone a synchronous change. In some cases where the mechanism has been fully realized, they have disappeared altogether. We, therefore, find in man a pattern picture much simpler than in his nearer biological relatives. Their inheritance has been proven by Bonnevie (9); very suggestive evidence had previously been offered by Wilder (2). The determination of the patterns cannot however be a direct or absolute one, since Cummins and Sicomo (10) (11) have shown that malformations of the hand and feet may modify or even suppress them entirely. We can harmonize this conflicting evidence if we assume that it is the form of the hands, feet, digits and apical pads which is determined by the germ plasm. Their growth is subject to modification by parakinetic factors in utero. The patterns and lines, being merely the result of the peculiar features of the pads and members, would be subject *indirectly* to a plastic inheritance. This hypothesis would explain not only the close relation between the patterns of a family group but also the frank and frequent exceptions to the demands of a strict inheritance.

In our examinations of M. T. we might hope to find that they and they only possessed dermatoglyphics identical in the form and the number of patterns and in the direction of the flow of the open fields. They might differ in minutiæ [2] and minor purely quantitative details. There are, however, several carefully studied cases in the literature which prove that individuals manifestly not M. T. may possess such prints. Waardenburg (13) describes two brothers, the one of whom was eight years older than the other, whose fingers were absolutely alike in their fundamental features, only slight differences were present and those were mostly quantitative, *i.e.*, differences in the number of lines which

[2] Term coined by Galton to describe small variations in the lines which do not alter the general picture of the pattern.

separated individual patterns. On one finger there was a small pattern below the crease of the last joint which was merely suggested and not completed in the homologue of the brother. For evidence concerning palm prints we can go to no better witness than to Wilder (2)—to whose conservatism in the use of the prints we must pay tribute. He tells of a father and son who had palm prints identical except for dimensional differences (Coll. 96). Case No. 99 is of still greater importance. The two individuals, a father and a son, had the same palm prints, which moreover were of a rare type and showed a suppression of the C triradius and an unusual form of the hypothenar pattern. In one of his earlier publications Wilder (*2a*) describes like sexed twins who were remarkably alike in color, figure, and features so that their best friends confused them. Their papillary prints were alike but their palm prints absolutely different. Wilder commented on this case: "It may also be possible to find a case of unlike fraternal (diovular—Ed.) twins with very similar palm formulas." This may be true of his two cases who were not "identical" to all observers but who had similar palm prints. Lauterbach (14) found two pair of different sexed twins in a mixed group of 74 pairs who fulfilled Wilder's conditions. The photographs of the prints in the one case shows such a remarkable similarity as is in the author's experience rarely found even in "identical" and most probably monoovular twins.

It should be particularly observed that we have attempted to quote only cases which can be classified without much doubt as either M. T. or D. T. With the exception however of sex, it is impossible to use any characteristic with any degree of certainty. We have no standard by which we can measure our diagnostic accoutrement and far too little time and thought have been spent in correlating the different methods suggested so as to clear this fog of uncertainty. There is, however, one group of twins about whose monzygotism there is no reasonable doubt. All authorities seem to agree that the conjoined twins and double monsters (cosmobii) represent the product of one ovum. The suggestion that such abnormalities are caused by a multiple fertilization has not gained many adherents. We refer the reader who is interested in this intricate question to Broman (15) and Wilder (*2a*) and (*d*).

The usual inking method for recording prints was useless, since all of our specimens had been in preserving fluids for years. We, therefore, sketched the patterns freehand with the aid of a binocular dissecting microscope (Leitz). Naturally such a method can lay no claim to strict quantitative accuracy. The patterns, however, were copied faithfully in respect to position and form and as no quantitative identity of the patterns was expected, this sufficed. The specimens were washed in running water for 12 to 24 hours, in some cases the tissues were softened in moderately hot water. Sometimes the superficial cuticle of the palms and soles had to be removed with a fine brush and pincette. The lines then stood out clearly on the fresh surface of the epidermis.

We have used the ingenious Wilder (2) formula in describing the palms and soles and the Galton scheme for the fingers. The modification of Wilder's method as applied to the palms which has been prepared by the painstaking labor of Cummins (16) has been of immense value to us. The attempt to visualize the formulas of earlier workers who used the simpler Wilder formula taught us the value of Cummins' work.

An examination of the prints and formulæ of Table I. reveals astonishing differences between these indubitably monoovular twins. In T. 61 we call attention to the similarity of the three hands and the dissimilarity of the fourth—the right hand of the right twin. By some authors this condition is considered to be quite frequent among same-sexed twins. It might, therefore, be of importance in the diagnosis of monoovularity. There is, moreover, a close relationship between the formulæ $9.\times 5.3$ and 10.7.6.5". In T. 144 the palms again show differences which are of only quantitative value, a shift of the radiant of D but a few lines distally would produce the same formula as is found in the left twin. In T. 60, however, we find in the absence of pattern B and the entire disappearance of Triradius D from the right palm of the left twin gross quantitative differences which together with the minor differences in the left palm of the left twin produce a picture which is indistinguishable from the palms of most different sexed and therefore dizygotic twins.

The sole prints are perhaps still more confusing. T. 60, which showed palm prints of great dissimilarity, has sole prints which

are asymmetrically very similar; *i.e.*, right corresponds to left. In T. 61 and T. 16 we find a similarity between the three members but striking differences in the fourth: in T. 61 pattern W takes the place of A in the right foot of the left twin, and in T. 16 the entire print of the right foot of the left twin differs from its homologe. T. 144 and T. 7 have sole prints that exhibit remarkable dissimilarity in all members.

In T. 70 complete finger prints were obtained. There is a very close relationship between all these prints. The one noteworthy feature is that the spiral character of the patterns on both thumbs of the right twin is not present in any of the patterns of the left twin. Bonnevie (9) believes that the shape of the pattern—circular or elliptical—and the twisting tendency of the ridges are hereditary and that they are perhaps even dominant characteristics. Our observation seems to speak strongly against the purely hereditary nature of the twist. Bonnevie expects to find these characteristics present in both of all M. O. twins.

Though these results were very disappointing, we did not believe that they eliminated dermatoglyphics as diagnostic aids. It might be that the coincidence in dermatoglyphic similarity between twins is much greater for the M. O. than for the D. O. It is, however, evident that the moment we abandon the hope of finding an exact identity—which of course we must do!—and speak of similarities, we have assumed a quantitative attitude toward the problem. Cummins [3] suggested a method which is perhaps of value in racial comparison but which proved quite useless in our study. The classification which we propose is not based on mensuration and therefore cannot exclude the element of personal judgment; it does however permit a correlation of the palm print with other methods of zygotic diagnosis. Until some more rigid scheme be discovered, it should prove of value.

Our prints and those found in the literature seem to fall into five classes which we have termed: (1) absolute identity, (2) apparent identity, (3) probable identity, (4) transitional, (5) non-identity.

I. *Absolute Identity*—Ab. I.

All four hands or two by two, either symmetrically or asymmetrically, are the same in formula and print. In the print small differences may

[3] Personal communication.

be present which are due to the minutiæ and which do not appear in the formula.

II. *Apparent Identity*—Ap. I.

All four hands or two by two, symmetrically or asymmetrically, are the same except for slight differences in the formula as well as the print which are however purely quantitative. Another type of difference found is that due to segregation: the appearance of some striking difference in one hand only, when the differences between the hands otherwise do not exceed the limits prescribed above. This is found particularly in the thenar and hypothenar patterns. The appearance of an anomolous characteristic on one side of one M. O. twin has been described for numerous other characteristics such as Darwin's node, etc.

III. *Probable Identity*—P. I.

Three hands are absolutely alike in formula and in print (except for minutiæ). The fourth hand reveals general similarity in the flow of the lines but has minor though radical differences. The group has been separately classified because Lauterbach (14) has been impressed by its prevalence among like sexed twins. (*e.g.*, Cosmobii T. 61.)

IV. *Transitional*—T.

Two by two, the same, symmetrically or asymmetrically, except for unimportant differences which are however not merely quantitative. The prints must show closely related forms. The frequency of this type of print in our group is most likely due to the inheritance of the family type.

V. *Non-Identity*—N. I.

All prints which show radical differences that are not related forms. There is no identity between any three hands as in P. I.

A correct classification of the palm prints can be undertaken only if the publications of Cummins (16), Midlo (16), Sicomo (10), etc., have been studied. Apparently different symbols often disguise a very close resemblance. For instance X, 0, 7 and 9 in the C position are often very similar, a mere shift of one or two lines may change the formula from the one to the other. In the same way 9 and 10 are only quantitatively different whereas a change from 10 to 11 would entail a change in the pattern B, etc.

In obtaining our material for the investigation of the palm prints of non-conjoined twins, we took pains to avoid selection of material and therefore examined all twins who came as patients to the ward or dispensary. Our technique of printing was similar to the ink method as described by Wilder (2) and Cummins (10), (11), (16). We found it important to ink the hand lightly; for this purpose we secured a marble slap and rolled out the printer's

ink (mimeograph) in a thin film with a rubber roller. The exact amount of ink which is used is of great importance. A double weight glazed paper greatly improved the clarity of the print. The printing itself is best done by wrapping the paper around a wood or glass cylinder about 6 cm. in diameter and rolling off the hand quickly and lightly, preferably from the distal end toward the wrist. Proper assistance is absolutely necessary when printing the palms of infants or young children. The assistant should hold the wrist firmly and bring the thumb into extreme abduction. The operator presses the four fingers flatly within his one hand and rolls off the print with the other. It was frequently necessary to secure an enlargened photograph of the print to elucidate the extremely fine lines of the young child. A negative will do for this purpose. Palm printing of infants is in every case difficult. Despite skill and care blurred prints will often be obtained which allow us to trace the general flow of lines and the position of patterns with certainty but which cause many dimensional errors. Our classification rests upon general similarities and not on identities. We, therefore, believe it is possible to utilize such prints.[4]

We obtained forty pairs of satisfactory prints. Our first observation was, that two different sexed twins—and therefore undoubtedly diovular—had prints of the class, Apparent Identity. Unfortunately the number of unlike sexed twins is too small (six) to reveal the frequency of such cases. We therefore resorted to a correlation with the results obtained by examining the twins according to Siemen's method (3).

The author has reserved for a second paper an exposition of Siemen's method and its limits of error. The diagnosis is made on the basis of identity in a number of different characteristics such as iris color, hair form, distribution and color, skin type and color, face form, lanugo distribution, etc. The probability that a pair of twins is monovular increases with the number of identities in genetically unrelated characteristics. It is essential that characteristics are chosen which show a wide range of variability. It then becomes increasingly improbable that any two individuals not possessing the same germ plasm would show such identity.

[4] We had less success with sole prints. It is likely that the Mathew method of sole printing would have improved our results. The formulation of sole prints meets with greater difficulties too.

This assumption is not forced; its theoretical statistical justification is the basis of the Bertillon system. It will suffice to say that the method has given satisfactory results in the hands of a number of observers.

The twins who have been hitherto examined however were all adults or older children. Our own investigation was confined to infants and young children. This may be the cause of the failure of the method in our hands. We were unable to obtain the sharp differentiation of M. O. and D. O. twins which other workers have apparently secured. For a further discussion of this point we refer the reader to our second paper. Case No. 24 does however offer a serious objection to the use of dermatoglyphics. These were twins who had the same skin type, iris, hair color and sex and whose skin color and lanugo distribution showed only slight differences. Seventeen of twenty-two major characteristics were identical in both twins. Yet their palm prints were absolutely dissimilar.

Such results are undoubtedly in contradiction to the popular conceptions of dermatoglyphics. Misled by the inheritance and the complicated structure of the patterns, we have assumed that they must necessarily be different in individuals of different germ plasm. We have failed to consider the rôle which purely parakinetic factors in utero must exert upon the form and growth of the primitive pads and hence upon the patterns. Cummins (10), (11), (16) has published a number of cases in which abnormal patterns have been found in association with syndactilism. We call attention to the bizarre pattern on the right foot of the parasite in our case T. 7. We may therefore conclude: (1) That like finger and palm form will lead to fundamental—though not detailed—similarity in patterns and (2) that parakinetic factors in utero will modify both. The late appearance of the ridges in the embryo of course increases the risk of such a modification.

It has been repeatedly suggested by various writers that a valuable criterion for the differentiation of M. O. and D. O. twins is the comparison of the differences which exist between the two halves of the individual twin with those that exist between the pair. In the M. O. twins the latter differences are supposed to be no greater than the former. In palm prints we have not found

this to be of any value. On the contrary Case 25 whose prints show almost symmetrical identity with obvious differences between the rights and lefts of each twin, had dissimilar iris color, lanugo distribution and skin color. Lauterbach's (14) case 101 shows the same type of formula. Here there can be no doubt about the zygotism as the pair was different sexed.

Only two other workers have examined the dermatoglyphics of a sufficient number of *unselected* twins.[5] None have correlated their results with Siemen's technique. Lauterbach (14) has published a number of cases of dizygotic twins who had identical palm prints. Montgomery (20) is more enthusiastic about sole prints. We believe that his own statistics show that they cannot have the absolute value which was at first accredited to them. Only twelve of his fifty-seven like sexed twins had like sole prints whereas his group—which was unselected—must have had about twenty-eight M. O. pairs.[6] In other words here, too, only half or less of the M. O. twins had similar sole prints. His different sexed twins show that similar sole prints are rare among dizygotic pairs. Bonnevie's work (9) is strangely discordant. Her ingenious method which permits an exact mensuration of finger prints has proven that these are dependent in large measure upon inheritance factors. Her examination of twins is of special interest to us. Fifteen pairs which she regards as undoubtedly M. O. had an r of 0.924 ± 0.037. Fraternal twins showed an r of 0.535 ± 0.082 and siblings of different sexes, 0.595 ± 0.118. The r of unrelated individuals was 0.27 ± 0.128. In other words we have a remarkable correlation of finger prints in M. O. twins which is not present in siblings. These statistics however obscure great individual variations. Among the siblings, for instance, we have 9 pairs out of thirty in which the percentage differences fall within the group of the M. O. twins though the arithmetic average of the percentage differences for the former is 28 whereas it

[5] Newman's article on the same subject which appeared in the Biological Bulletin of October, 1928, came to our attention too late for discussion in this paper. We shall discuss it in our second article.

[6] According to Weinberg's differential method (18) (19) in any unselected group of twins, about ⅓ of all twins and about ½ of all like sexed are M. O. In small groups the figures are naturally merely approximately correct. Recent statistics and all theoretical conclusions justify Weinberg's assumptions.

Table I.

T. 60. Duplicitas asymmetros. Thoracopagus. Tetrabrachius.
Palm Prints: Right Twin, (R.) 10.7.6.3/B.O.O.Ac.L.C13.
(L.) $10.^{87}.^{96}3$/B.O.O.Ac.L.C12.
Left Twin, (R.) 10.0.6.3/O.O.O.Ac.Z.C12.
(L.) 9.7.5′.3/B.O.O.Ac.L.$C12^{3}$.
Classification—Transitional.
Sole Prints: Right Twin, (R.) (U + U + U + U) od.
(L.) (U + U + U) + U. ld.
Left Twin, (R.) (U + U + U + U) od.
Finger Prints: Right Twin, (R.) SW—RL—UL—UL—UL.
(L.) SW—RL—UL—UL—UL.
Left Twin, (R.) W—UL—UL—UL—UL.
(L.) UL—RL—UL—UL—UL.

T. 16. Duplicitas symmetros. Dicephalus. Dibrachius. Dipygus.
Sole Prints: Right Twin, (R.) BC—(U + U + U) od.
(L.) BC—(U + U + U) od.
Left Twin, (R.) B—U + (U + U) ld.
(L.) BC—(U + U + U) od.

T. 61. Cephalothoropagus, Syncephalus Asymmetros.
Palm Prints: Right Twin, (R.) 10.7.6.5″/O.OO.Ac.L.C13.
(L.) 9.X.5″.3./O.O.O.Ac.Ac(C13).
Left Twin, (R.) 9.X.5.3/O.O.O.Ac.Ac.C13.
(L.) 9.X.5.3/O.OO.Ac.Ac.C13.
Classification—Probable Identity.
Sole Prints: Right Twin, (R.) A—(U + U + U) d'meet.
(L.) A—(U + U + U) d'meet.
Left Twin, (R.) W—(U + U + U) d'meet.
(L.) A—(U + U) + O d'in.

T. 7. Duplicitas asymmetros, Autosite et Parasite. Thoracopagus asymmetros. Epigastrius. Cyclopia.
Sole Prints: Autosite, (R.) A—O + U + O. ld.
(L.) A—O + (U + U). ld.
Parasite, (R.) O—O + O + O. hy. od.
(L.) AB—(U + U + U) ld.

T. 144. Cephalothoracopagus asymmetros
Palm Prints: Right Twin, (R.) 8.6.5′.3./O.O.O.O.L.C12.
(L.) 8.6.5.3/O.O.O.O.L.C12.
Left Twin, (R.) 7.5.5′.3/M.O.O.O.L.C13.
(L.) 7.5″.5.3/O.O.O.O.L.C11.
Classification—Apparent Identity.
Sole Prints: Right Twin, (R.) A—O. + ∩. + U. 2d.
(L.) A—O + ∩ + U. 2d.
Left Twin, (R.) A—O + (U + U). 1d.
(L.) A—O + O + ∩. 2d.

Legend: We refer the reader to Montgomery (20) for formulation of sole prints. The parentheses include patterns which are apparently united in one. The finger print formula is the common one of Galton. S.—Spiral. W.—Whorl. L.—loop opening radially, (R.) or ulnarly (U.).

The reader is referred to the work of Cummins and Wilder for the

is only 3.5 for the latter. As siblings are the genotypic equivalents of fraternal twins, we may assume that Bonnevie's method can be of no use to us in the diagnosis of individual pairs of twins. Possibly a large group of unselected twins may show that M. O. twins never or rarely have a high percentage difference. In that case Bonnevie's method would be a valuable negative trait; that is to say the presence of a high percentage difference would exclude M. O. Bonnevie's study suggests this conclusion. We wish to call attention to the numerous cases of extremely small percentage differences among siblings whereas the groups of fraternal twins show a general high level. We believe that this is due to sampling; inevitable, as Bonnevie was not working on the same problem as we are. Her experience, however, illustrates how necessary it is in research along this line to avoid artificial and undesired selection.

Conclusions.

A method of classifying palm prints is suggested. Forty pairs obtained on non-conjoined twins were correlated with the results obtained by Siemen's method of diagnosis. No strict parallel between the identities in skin color, iris color, hair color, lanugo distribution, etc., and the similarities in palm prints could be found. The correlation of the degree of similarity with the sex of the pair suggests that palm prints are of little value in selecting monoovular twins. The study of palm, sole and finger prints in conjoined monsters leads to the same results.

We believe that characteristics of value in the diagnosis of monoovularity can be objectively chosen and their exact nature and evaluation elucidated only by studying unselected groups of like and unlike sexed twins indiscriminately.

The writer wishes to express his sincere appreciation to Professor T. Wingate Todd and to Professor N. William Ingalls for the permission to use the specimens of The Hamann Anatomical Museum and for much valuable advice.

formulation of the palm prints. Slight changes made by the author are as follows: *Ac.* = arch. *L* = Loop: small loop represented by small *l*. *O* = no pattern. *hC* = high carpal pattern. *d* = extra triradii distal to Y of a pattern with index number showing position on palm. When capital (D) the extra triradius is peripheral to Y. *Z* = missing pattern due to lack of triradius at that point. *X* = triradius has a straight Y. Doubtful readings are represented by a circle about the symbol.

LITERATURE.

1. (*a*) **Galton.** Inquiries into Human Faculty and its Development. Macmillan, London, 1883.
   (*b*) **Galton.** Finger Prints. Macmillan, London, 1892.
2. **Wilder.** (*a*) American Journal of Anatomy, 3, 387, 1904; 1, 423, 1902.
   (*b*) Anatomischer Anzeiger, 32, 193, 1908; 13, 250, 1897.
   (*c*) Journal of Heredity, 10, 410, 1919.
   (*d*) Biological Bulletin, 30, 135, 1916; 30, 211, 1916; 50, 393, 1926.
   (*e*) A. J. of Phys. Anthropology, 5, 143, 1921.
3. **Siemens.** Zwillingspathologie. Verlag Springer, 1924; Virchows Archiv, 253, 746, 1924; 263, 666, 1927.
4. See discussion between *Siemens* and *Meirowsky*. Virchows Archiv, 266, p. 313, 1927.
5. **Just.** Die Biologie der Person Brugsch-Lewy. Urban-Schwarzenberg, 1926, No. II.
   Lenz, also **Bauer.** Handbuch der normalen u. path. Physiol., XVII. (III.). Springer, 1926.
   Siemens. Konstitutions- u. Vererbungspath. Springer, 1921, p. 76.
6. **Schlaginhaufen.** Morph. Jahrbuch, 33, 577, 1905; 34, 1, 1905.
7. **I. Whipple (Mrs. Wilder).** Zt. f. Morph. u. Anthrophol., 7, 261, 1904.
8. **Schultz.** Zoologica, III., No. 12.
9. **Bonnevie.** J. of Genetics, 15, 7, 1924.
10. **Cummins & Sicomo.** Anat. Record, 25, 355, 1923.
11. **Cummins.** Anat. Record, 26, 1, 1923.
    Cummins. A. J. of Anatomy, 38, 89, 1926–27.
12. **Poech.** Mitt. Anthro. Ges. Wien., 1925, LV., 133–159, abst. in A. J. of Phys. Anthrop., 9, 384, 1926.
13. **Waardenburg.** Klin. Wochenschrift, 5, 2115, 1926.
14. **Lauterbach.** Genetics, 10, 525, 1925.
15. **Broman.** Normale u. abnormale Entwickelung des Menschen Bergmann, 1911.
16. **Cummins & Midlo.** A. J. of Phys. Anthropol., IX., 471, 1926.
    Cummins et alt. A. J. of Phys. Anthropol., 11, No. 3, 1928.
17. **Verschuer.** Ergeb. der Inn. Med. u. der Kinderheilkunde, 31, 35, 1927. (Has extensive literature!)
18. **Weinberg.** 1901—discussed in Newman's Biology of Twins (U. of Chicago Series), 1917. Literature in Verschuer (q.v.).
19. **Dahlberg.** Twin Births and Twins from a Hereditary Point of View (in English). Stockholm, 1926, Bokfoerlags—A. B. Tidens Tryckeri.
20. **Montgomery.** BIOL. BULL., 50, 293, 1926.

MUSCULAR REORGANIZATION IN THE ODONATA DURING METAMORPHOSIS.

ARTHUR D. WHEDON,

DEPARTMENT OF ZOÖLOGY, NORTH DAKOTA AGRICULTURAL COLLEGE.

INTRODUCTION.

Extensive investigations have been made into tissue reorganization in the Holometabola, especially the Lepidoptera, Diptera and Hymenoptera. The profound nature of these changes has probably overshadowed the less extensive ones which might be seen in the Hemimetabola. At least, no study has been devoted to the latter. Indeed, so little is known of the Neuropteroid and Orthopteroid insects in this respect, and perhaps so much inferred from the Holometabola, that a gross misconception has persisted almost to the present regarding even the period in the life cycle of such an insect when tissue reorganization is accomplished, to say nothing of uncertainty as to the nature of the metabolic processes responsible for the changes. The Odonata, perhaps typical of those forms which pass rather suddenly from aquatic larva to aerial imago, is a good example.

In his "Biology of Dragonflies," Tillyard (1917) states that "The emergence of the imago from the larval skin or exuviæ, usually spoken of as metamorphosis, is in point of fact only the consummation of an internal metamorphosis which begins a considerable time before. The beginning of this change is marked by an alteration in the color and behavior of the larva. The color darkens considerably, greenish larvæ becoming a dull opaque brown. The larva becomes listless and refuses to feed. Rapid proliferation of the hypodermal cells, preparatory to the formation of the imaginal exoskeleton, causes the larva to appear tense and swollen." The changes in the eyes and other parts are then mentioned but nothing is said of the retraction of the labium, which begins at the time the insect ceases to feed, or of the degeneration of the gills within the rectum and of the opening of

the thoracic spiracles at the time the larva comes to the surface. Then Tillyard continues "As soon as the changes are practically complete, the larva climbs out of the water, usually upon a stick, rock, reed-stem or other suitable object." A description of the final ecdysis follows.

As early as 1918 the writer observed considerable variation in the structure of the adult abdomen in the Odonata (Whedon, 1919), especially in *Anax*. Specimens which had been collected at various times without reference to their exact ages, when dissected showed now the presence and again the absence of certain muscles in a way very puzzling to one possessed of the idea that all reorganization had been completed before transformation. It later became clear that newly emerged specimens (tenerals) retained wholly or in part the larval structures. In 1924 Miss Ford (Ford, 1924) also discovered this and briefly discussed the condition in the muscles of *Libellula quadrimaculata*. She, further, gave a homology of the abdominal muscles of the larva and the adult based upon her findings. So much, however, remained vague or unknown that the present study was deemed worth while.

Materials and Methods.

Most of the work which follows was done upon *Anax junius*, although *Libellula* and *Sympetrum* have occasionally served for comparison. Specimens were collected and prepared at Woods Hole and Fargo during the seasons between 1919 and 1928. They were usually decapitated, opened, spread in a wax tray and immediately fixed. Bouin's Fluid, Zenker-formol, 10 per cent. formalin and 95 per cent. alcohol were used. Other specimens were injected with methylene blue and fixed in ammonia molybdate in an attempt to stain the nerve branches leading to muscles in different conditions. While not very successful for nerve endings, these methylene blue preparations were excellent for dissection.

The dissections were made under a Greenough binocular in a paraffin tray with translucent bottom, thus permitting of both reflected and transmitted light. The specimens were kept immersed in the preserving fluid.

Muscles and other tissues were also imbedded in paraffin or

celloidin, sectioned and stained with iron-hæmatoxylin to determine the normal or pathological conditions at various stages during metamorphosis. This paper makes no attempt, however, to discuss the cytological and histological changes in muscular tissue during its degeneration.

The author wishes to acknowledge the kind assistance of Doctor Philip P. Calvert of the University of Pennsylvania, especially in connection with the bibliography and the loan of papers. The Academy of Natural Sciences of Philadelphia, also, very generously furnished photographic copies of Rogozina's plate of the nerve branches of an abdominal segment of *Æschna*.

The Essential Conditions of Transformation.

Transition from a well adapted aquatic larva to a highly specialized aërial adult does not require profound changes in all of the animal's systems. At least, this is true so far as superficial anatomy is concerned. The nerve chain, the heart, the Malpighian tubules, and the gonads develop quite directly and progressively from embryo to adult, though the histology of the ganglia, the atrophy of nerve branches and formation of new ones would be expected to adjust themselves to the reorganization of the digestive and muscular systems. On the other hand, the alimentary canal, the tracheæ and their air sacs, the fat body, and the muscles are extensively modified. The greatest change in the alimentary canal is due to the relatively sudden loss by the rectum of its respiratory function, resulting in this organ becoming as inconspicuous in the adult as it is remarkable in the larva. This rectal modification leads in turn to the disuse of the large tracheal trunks and branches which supplied it, the necessity of opening the spiracles, first the thoracic and then the abdominal, and the development or enlargement of numerous air sacs. New thoracic muscles must be built to equip the wings, but the heavy musculature of the abdomen, correlated with respiration and locomotion, must be greatly reduced.

The time necessary for the formation of new muscles is, of course, much greater than that for the atrophy of the superfluous ones, and thus the muscles for the wings appear gradually from instar to instar during larval growth (Poletaiew, 1881). The

degeneration of the abdominal muscles was thought, as stated earlier, to be accomplished while the larva was in the quiescent stage just preceding transformation but is shown by this study to occur mainly, if not entirely, after the emergence of the imago. The period of emergence itself is very short, scarcely ever exceeding an hour (*Anax, Plathemis* and *Sympetrum*), and is occupied with the more physical necessities of expansion.

The Degeneration of Abdominal Muscles.

While many stages have been dissected and sectioned for the determination of the muscle condition, the following will be sufficient for a clear demonstration of what takes place: (*a*) the normal fully grown larva, (*b*) the quiescent larva with retracted labium, (*c*) the transforming larva, (*d*) the newly emerged imago, and (*e*) the imaginal stages at about three, eight, eighteen, thirty-seven, sixty and eighty-three hours.

Full descriptions and figures of the muscles of *Lestes, Anax, Libellula* and *Tramea* have been given by the writer in an earlier paper (Whedon, 1919). A comparison of the abdominal muscles of a normal larva of *Anax* with those of a larva with retracted labium reveals no macroscopic differences. Under the binocular microscope the muscles are compact and functional in appearance (Fig. 1). Sections at this stage stained either in methylene blue or iron-hæmatoxylin show no distinct signs of degeneration. Teased muscles seem perfectly normal and are possessed of what seems their usual plentiful supply of tracheæ and nerves. These muscles must function actively, also, for when a larva is disturbed it swims vigorously by means of rectal contractions and abdominal movements. Perhaps this could not continue to the moment of emergence, however, due to the changes in the respiratory system.

Dissection of a specimen well along in this stage shows that the gill system of the rectum is breaking down, the lining is being shed and the tracheal connections with the wall of the rectum are degenerated though still to be seen. It is the progress of this degeneration that forces the larva to the surface of the water to breathe, first through the rectum and later through the thoracic spiracles. Such larvæ drown if kept under the surface of the

water. Specimens not too far gone, have been resussitated after respiratory movements have ceased, by gentle manipulation in the air. Dissection shows the tracheal trunks and branches identical with those of the full-grown larva except for the connections with the rectum and the beginnings of air sacs. Many of these tracheæ run quite parallel to the main nerves. Teased fragments and sections of tergal and sternal muscles show large numbers of tracheoles, apparently in functional connection with the fibers.

The nervous system and its neuromotor connections also seem indistinguishable from those of the normal larva. The nerves of a central abdominal segment have been dissected out in a fully grown larva, in a larva with retracted labium, and in a specimen in the act of emerging. The nerves are identical except for such changes in position as result from the elongation of the abdomen (Fig. 9). The more minute branches have not been followed in many cases. Efforts to use methylene blue intra vitam have not met with pronounced success, so that the nature of the nerve junctions with muscles about to degenerate is not known with certainty.

Specimens fixed by different methods during transformation all show the same condition of the muscles. In dissections they seem identical with those of the normal larva, with no outward sign of degeneration (Fig. 9). Longitudinal sections of the whole body wall with its muscles in place when stained in iron-hæmatoxylin give little or no evidence of general disintegration in the large sternal and tergal muscles. Fibers in various states of contraction (Jordan, 1919, 1920) are present. A few muscles and portions of muscles, however, show a flaky condition and lack of affinity for stain which is not easy to interpret; their nuclei are still normal though perhaps slightly smaller than usual. Something similar to these conditions may be seen, however, in a highly contracted normal muscle fiber.

At this stage the rectum has contracted to nearly its final size and condition, the lining, together with the remnants of the rectal gills, has been shed and the surrounding tracheæ are largely disintegrated.

Special attention was given to the nervous system of the transforming larva and of the newly emerged imago in the hope of

learning the relations of the nerve branches with the muscles doomed to degeneration. Dissection showed minute nerves penetrating these muscles, though, as stated earlier, nothing conclusive has been determined with respect to their neuromuscular junctions proper. An attempt to show the nerve distribution is made in Fig. 9. The literature contains little of an accurate nature respecting either the distribution or the nomenclature of the nerves of the abdominal segments. Rogozina (1924) has sketched in much detail the distribution of the nerves and their branches of the right side of an abdominal segment of the larva of *Æschna*. Unfortunately the figure does not make clear the more exact relations of these nerves to the various muscles. Many of the larger branches, also, do not occur as in *Anax* (Fig. 9) and there are many variations in the smaller branches. The unpaired ventral nerves she omits entirely. The three main lateral nerves from the ganglion she designates, as do all other authors consulted, N_1, N_2 and N_3. Reference to the excellent figures in Zawarzin's histological paper (1924) and to Fig. 9 below will clearly establish their relations. The remainder of Rogozina's labels are in Russian and have not been translated, so exact descriptions must here be omitted. Perhaps the greatest differences between Rogozina's figure and Fig. 9 are to be found in the branches of N_3. She shows no branching until this nerve has run caudad and laterad to the pleural region, while in *Anax* repeated dissections show it to run directly caudad to a point slightly beyond the origin of the Tertiary Longitudinal Sternal Muscle (*tls*) where it divides into ventral and dorsal branches of about equal size. The former passes transversely beneath the bases of the Tertiary and the Quaternary Longitudinal Sternal Muscles (*tls* and *qls*) to the pleural region where it supplies the Median Dorso-Ventral Muscle (*dvm*). The dorsal or more internal branch passes over the ends of the muscles named (*tls* and *qls*) and on to the pleural region where it joins a branch of N_2 on its way to the Dorso-Ventral Oblique Muscle (*dvo*) and perhaps the other muscles and hypodermis of this region.

There is no agreement as to the functional nature of the three main pairs of nerves from each abdominal ganglion. To the writer, they all seem to be mixed nerves. This accords with the

results of Zawarzin (1924) but differs from those of Rogozina, in Odonata, and of Hilton (1924 and 1925) and others in Coleoptera and other insect groups. N_1, the largest and most anterior of the three, is certainly a mixed nerve. It can be plainly seen to be made up of two branches confluent at about the point of crossing the Dorso-Ventral Oblique Muscle (*dvo*). The anterior and more dorsal branch carries the fibers from innumerable nerve endings in the hypodermis of the tergum, while the more posterior and ventral one is made up of the fibers from nerve endings in all of the tergal muscles: from those which are to degenerate as well as those to persist in the imago. N_2, the second nerve, seems to have a similar constitution but to supply the pleural region. Basal branches are apparently concerned with the muscles and hypodermis of the sternal region. The distribution of the divisions of N_3 have been described above.

Neither dissections or sections give evidence of a degenerating nerve supply to the Primary and Secondary Sternal and Tergal muscles. If such exists it is in the neuromuscular end-plates and here further investigation is necessary.

A comparison of the retracted labium stage and the transforming stage makes it certain that (1) there is little if any degeneration of the muscles preceding the imago, (2) there is still a full tracheal supply to the muscles, though that to the rectum has atrophied, and (3) nerve branches still connect with all of the muscles. This, of course, refers primarily to the abdomen.

From emergence on through many hours degeneration of superfluous larval structures continues. Adjustment and coördination of imaginal organs occurs at the same time. The length of this period varies with different genera. As determined by dissections, the reorganization seems completed in *Anax* in seventy-five to eighty hours, but *Libellula* at four hours has reached about the same condition as *Anax* at eighteen. *Plathemis* is about like *Libellula*. Miss Ford records a similar condition in *Libellula quadrimaculata* at three hours.

The grosser indications of this change in the muscles are an increasing flaky or granular appearance and the formation of oil droplets in both the muscles and the atrophying fat-body, together with the disappearance of the tracheoles and nerve branches. At

eight to sixteen hours the nerves to the degenerating muscles are gone while those to the remainder are very clear. Sections of degenerating muscle stained in iron-hæmatoxylin show first a lack of affinity for the stain and loss of striations, then later disappearance of the nuclei, irregularity in staining and a lumpy segregation which no longer yields histological detail. The sternal and tergal muscles go at the same time.

The progress of muscle degeneration with increasing age shows clearly in dissections made at eighteen, thirty-seven, sixty and eighty-three hours, the fifth segment being used in each case for examination (Figs. 5, 6, 7 and 8). By sixteen to eighteen hours in *Anax* (three to four in *Libellula*) the degenerating muscles are all distinctly granular and yellowish, though the forms of the muscles and muscle fibers are still retained and are of nearly their original bulk. At thirty-seven hours these have been reduced to a thinner sheet, more or less broken and perforated, and with the identity of the fibers nearly gone. At sixty hours but a thin, broken or lacy film remains, and at seventy-five to eighty-five hours the last traces of even this have disappeared, leaving only the marks of attachment on the skeleton to define their original positions.

The dissection of a single specimen of *Anax* at an age between eighteen and twenty-four hours yields a sequence in degeneration in the segments from anterior to posterior quite comparable to the progression just stated. When the muscles of segment 5 are at the stage shown for eighteen hours, segment 1 approximates the forty hour condition, while segments 7 and 8 are just beginning to possess a distinctly granular structure (Figs. 2, 3, or 5, and 4). Thus traces of certain muscles will remain in the posterior segments of the abdomen for some time after most of the segments have reached the adult condition.

No new muscles develop in the abdomen as the insect passes through the transformation period, except perhaps those of the copulative organs. The adult musculature is, in the main, the remnant of that of the larva. When all degeneration is completed the muscles which remain are the Tertiary, Internal Tertiary and Quaternary Longitudinal Sternals; the Tertiary, Quaternary, Quinary and Sextic Longitudinal Tergals; and the Anterior and Pos-

terior Dorso-Ventral Muscles. With the lengthening of the abdomen after emergence certain of these muscles which lay near together in the larva are drawn so close as to overlap or combine to form the apparently single adult muscles. It is clear that the three pairs of Longitudinal Sternals of the larva thus combine to form the Longitudinal Sternal muscles of the adult, and the Tertiary and Quaternary Longitudinal Tergals unite to form the Superior Longitudinal Tergals. *Anax* at eighteen hours gives indications of these in process of union (Fig. 3). The Sextic Longitudinal Tergals form the Inferior Longitudinal Tergals, and the Quinary Longitudinal Tergals, lengthened and reduced, become the Tergo-Pleurals (*tp*). The anterior and posterior Dorso-Ventral muscles remain and retain the same names in the adult.

In her paper on the abdominal musculature of Orthopteroid insects, Miss Ford quotes (pages 255 and 256) the writer's work of 1919 regarding the difficulty of homologizing the larval and adult muscles, and follows up her discussion with a table listing the homologous muscles. Comparison will show that her conclusions do not agree with those here stated. The present results are, however, based upon many and repeated observations over a period of several years and it is hoped they will be found correct.

The chief purpose of this short paper has been to establish the facts regarding the outstanding changes in musculature during metamorphosis in order to lead to the more fundamental problems of the histology, cytology and physiology of muscle histogenesis and degeneration in the Hemimetabola.

Summary.

1. There has long existed a misconception as to the time and nature of tissue and organ reorganization in the metamorphosis of the Hemimetabola. It is now determined that most of the changes in the muscles occur after emergence.

2. New thoracic muscles, ultimately to serve the wings, are added from instar to instar during the growth of the larva, and are carried over into the adult stage. The full complement of larval abdominal muscles is present through all the later instars and no new muscles are developed during transformation.

3. The heavier inner layers of larval abdominal muscles (the

Primary and Secondary Longitudinal Sternals and Tergals, the Middle Dorso-Ventral Muscles, and the Dorso-Ventral Oblique Intersegmental Muscles) degenerate soon after transformation. The outer and much lighter set remains to function in the adult.

4. For a short time after emergence the muscles which are to degenerate retain their normal appearance and are well supplied with tracheæ and nerves. Later they become yellowish and granular and gradually disappear. The time at which degenerative changes become noticeable varies with the genus, being early in *Libellula, Plathemis* and *Sympetrum* and much later in *Anax*.

5. Degeneration begins in the first segment of the abdomen and proceeds gradually to the last. It is completed in *Anax* in about three days.

6. The facts recorded make possible a statement of the homologies between the larval and adult abdominal muscles.

BIBLIOGRAPHY.

Cuvier, Georges.

1836–1846 Le Regne Animal. Les Insectes. 4 vols. Paris.

Deegener, P.

1912–1913 Nervensystem, Sinnesorgane. Handbuch Entom. (Schröder), Bd. I., p. 76–233, 77 figs.

Dufour, Leon.

1841 Recherches Anatomiques et Physiologiques sur les Orthopteres, les Hymenopteres et les Neuropteres. Mem. Math. Savants Etrangers, Acad. Sci. Paris, 1841, Tome VII., pp. 265–647, pls. 1–13.

1852 Etudes Anatomiques et Physiologiques, et Observations sur les Larves des Libellules. Ann. Sci. Nat., 3e Serie, Tome XVII., pp. 56–110, pls. 3–5.

Ford, Norma

1924 A Comparative Study of the Abdominal Musculature of Orthoperoid Insects. Trans. Royal Canad. Inst., Vol. XIV., Part II., pp. 207–319, pls. VII.–XXIII.

Hilton, William A.

1924 Afferent and Efferent Pathways in an Abdominal Segment of an Insect. Jour. Comp. Neur., Vol. 36, No. 3, pp. 299–308, 8 figs.

1925 Nerve Endings in Insects. Trans. Am. Micro. Soc., Vol. XLIV., No. 3, pp. 132–137, pl. IX.

Jordan, H. E.

1919 Studies on Striped Muscle Structure. V. The Comparative histology of the leg and wing muscles of the Mantis, with special reference to the N-discs and Sarcosomes. Anat. Rec., Vol. 16, no. 4, pp. 217–246, pls. 1–3.

1920 Studies on Striped Muscle Structure. VI. The Comparative Histology of the Leg and Wing Muscles of the Wasp, with special reference to

the phenomenon of the stripe reversal during contraction and to the genetic relation between contraction and intercalated discs. Am. Jour. Anat., Vol. 27, no. 1, pp. 1–68, 48 figs.

Leydig, Franz.

1864 Vom Bau des Thierischen Körpers. Handbuch der Vergleichenden Anatomie. Erster Band, pp. VI., 278. Tubingen.

Morrison, Guy D.

1927 The Muscles of the Adult Honey-bee. Q. J. M. Sc., Vol. 71, Part III., pp. 395–463, 12 figs.

1928 Ditto, Part II. Q. J. M. Sc., Vol. 71, Part IV., pp. 563–651, 41 figs.

Poletaiew, Nicolas.

1881 Du Developpement des Muscles d'Ailes chez les Odonates. Horae Soc, Ent. Ross., Tome XVI., pp. 10–37, pls. IV–VIII.

Rogozina, M. C.

1924 Die peripheren Nerven der Aeschnalarven. 1. Die Nerven der Abdominalsegmente. Bull. Inst. Recherch. Biol. Univ. Perm., T. 3. p. 141, 1 Taf. Paper in Russian; " Resume " in German.

Tillyard, R. J.

1917 The Biology of Dragonflies. Cambridge Univ. Press.

Viallanes, H.

1884 Anatomie et Dissection de la larve de Libellule. Feuille des jeunes Naturalistes, Tome XIV., pp. 81–87, pl. II. Paris.

Whedon, Arthur D.

1919 The Comparative Morphology and Possible Adaptation of the Abdomen in the Odonata. Trans. Am. Ent. Soc., Vol. XLIV., pp. 373–437, pls. XXI.–XXIX.

1927 The Structure and Transformation of the Labium of *Anax junius*. Biol. Bull., Vol. LIII., no. 4, pp. 286–301, 2 pls.

Zawarzin, Alexius.

1912 Histologische Studien über Insekten. II. Das sensible Nevensystem der Aeschnalarven. Zeitschr. Wiss. Zool., Band 100, pp. 245–286, 3 Taf., 9 figs.

1924 Uber die histologische Beschaffenheit des unpaaren ventralen Nervs der Insekten. Zeitschr. Wiss. Zool., Band CXXII., pp. 97–115, 5 figs.

1924 Zur Morphologie der Nervenzentren. Das Bauchmark der Insekten. Ein Beitrag zur vergleichenden Histologie (Histologische Studien über Insekten VI). Zeitschr. Wiss. Zool., Band 122, pp. 323–424, 8 figs., Taf. III.–X.

DESCRIPTION OF FIGURES.

All drawings were made with the camera lucida and Greenough binocular by the author. The magnification is approximately ten diameters unless otherwise noted. All dissections were pinned out flat in a tray. Healthy muscles are shaded with parallel lines, while those undergoing degeneration are stippled. The extent of degeneration is shown, roughly, by loss of definite outlines and lighter stipplings. Abbreviations used in labeling are as follows:

ag—abdominal ganglion.
car—mid-dorsal carina.
dva—dorso-ventral segmental muscles, anterior part.
dvm—dorso-ventral segmental muscles, middle part.
dvp—dorso-ventral segmental muscles, posterior part.
dvo—dorso-ventral oblique intersegmental muscle.
lpsp—lateral primary longitudinal sterno-pleural muscle.
N_1, N_2, N_3—first, second and third pairs of lateral nerves from abdominal ganglion.
nc—nerve cord.
N_1m—motor branch of N_1.
N_1s—sensory branch of N_1.
nmu—umpaired nerve.
pls—primary longitudinal sternal muscle.
qlt—quaternary longitudinal tergal muscle.
qnlt—quinary longitudinal tergal muscle.
sp—spiracle.
slt—secondary longitudinal tergal muscle.
sxlt—sextic longitudinal tergal muscle.
tls—tertiary longitudinal sternal muscle.
tlt—tertiary longitudinal tergal muscle.
trs—transverse sternal muscle.

PLATE I.

FIG. 1. Right half of abdominal segment 6 of a larva with retracted labium. All muscles are present and apparently in functional condition. Organs other than the muscles and main nerve cord have been removed.

FIG. 2. Left half of tergum of segment 2 of an imago eighteen hours after transformation. The stippled muscles are extensively degenerated, others normal.

FIG. 3. Sternum and right tergum of segment 5 of another imago eighteen hours after emergence. The heavier degenerating sternal muscles are dissected away on the right side. The overlapping and uniting of the Tertiary and Quaternary Longitudinal Sternals to form the Longitudinal Sternals of the adult, and a similar condition in the tergal muscles, can be seen. Nerve branches are not shown in detail. The degeneration of the larval muscles has not gone as far as in segment 2. Compare with Fig. 5.

FIG. 4. Left side of tergum of segment 7 of the same specimen shown in Fig. 1. The larval muscles show still less disintegration than in segment 5.

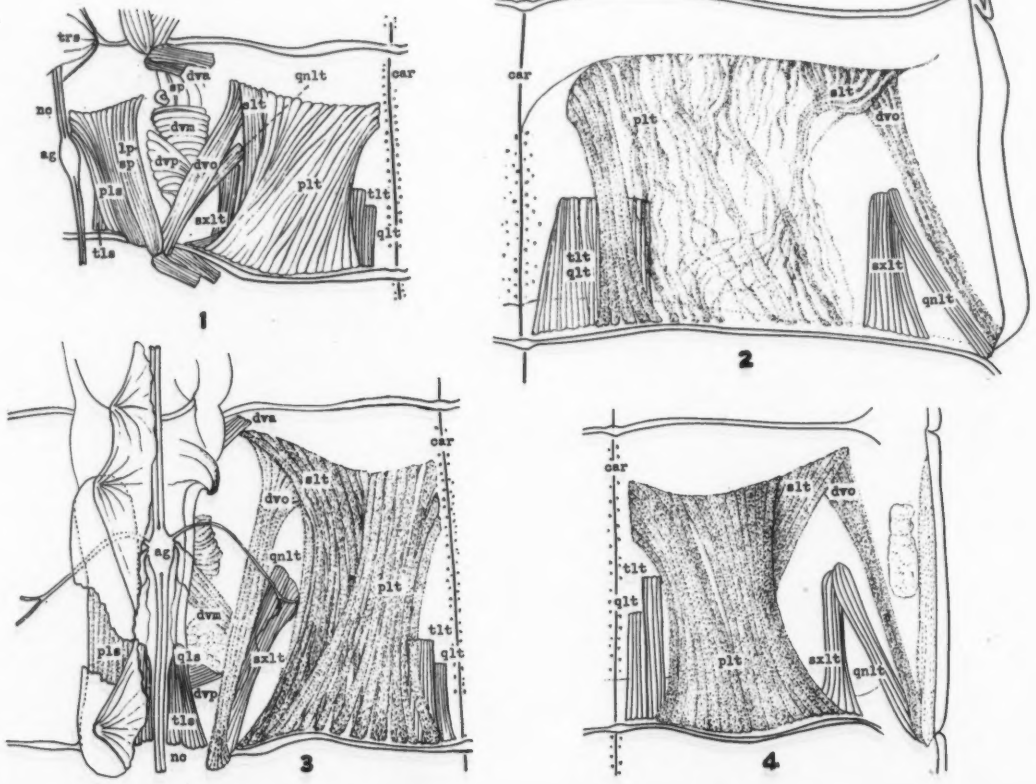

trs
dva
sp
qnlt
car
nc
slt
dvm
ag
lp
sp
dvp
dvo
plt
pls
tlt
sxlt
qlt
tls
1
car
slt
dvo
plt
tlt
qlt
sxlt
qnlt
2
dva
car
slt
dvo
ag
qnlt
plt
dvm
tlt
qlt
pls
qls
sxlt
dvp
tls
no
3
car
slt
dvo
tlt
qlt
plt
sxlt
qnlt
4

PLATE II.

All figures on this plate are of dissections of the left half of the tergal portion of the fifth abdominal segment at various ages after emergence. Taken together with Figure 1 they show the progress of disintegration of superfluous larval muscles from the normal condition to complete disappearance.

FIG. 5. Eighteen hours.
FIG. 6. Thirty-seven hours.
FIG. 7. Sixty hours.
FIG. 8. Eighty-three hours.

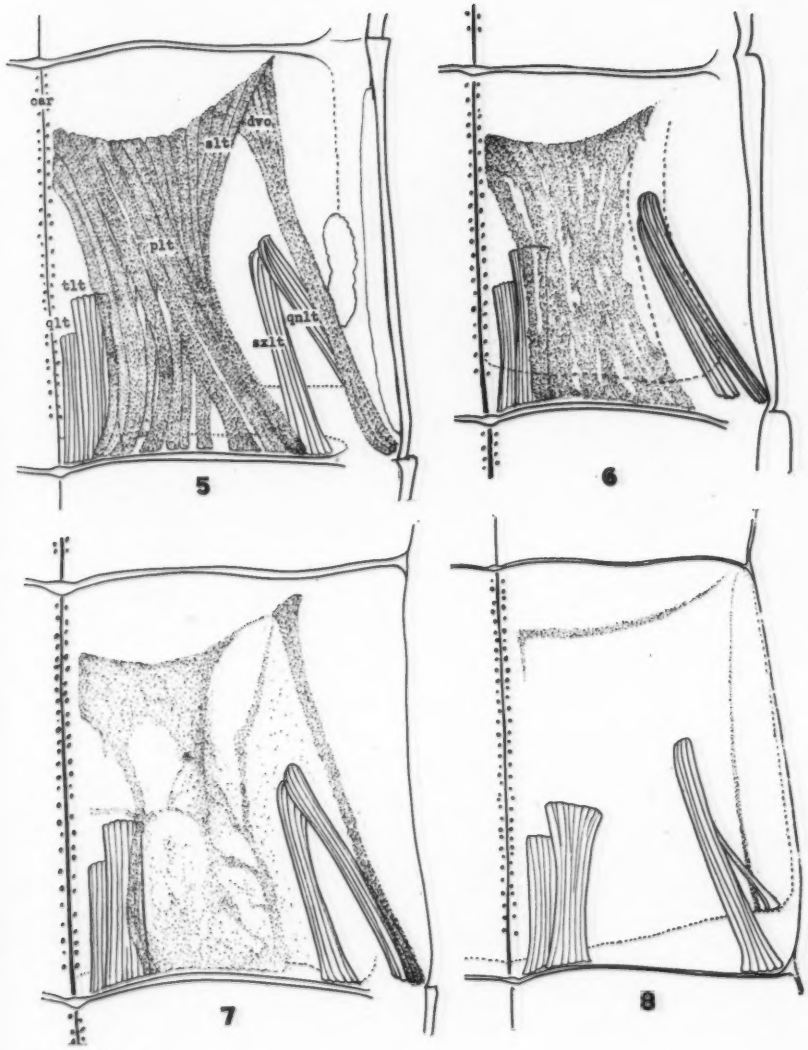
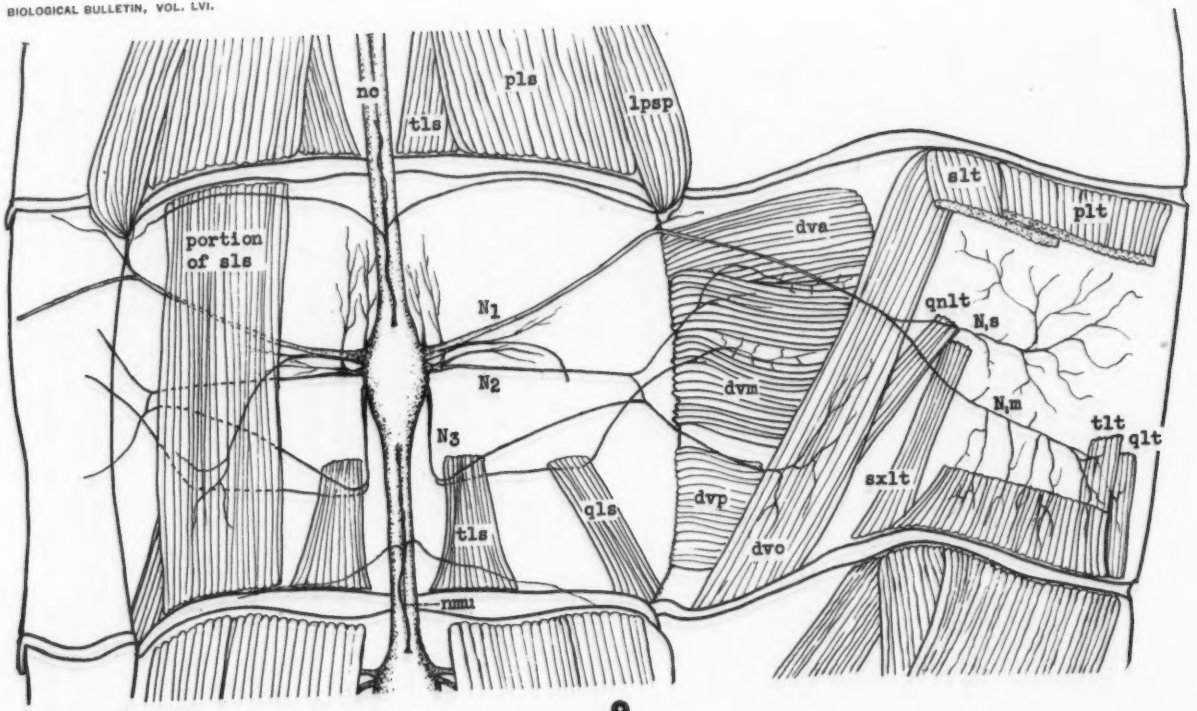

car
alt
dvo
plt
tlt
qlt
qmlt
azlt
5
6
7
8

PLATE III.

FIG. 9. Sternum and right half of tergum of abdominal segment 7 of a specimen injected during emergence with methylene blue, fixed in ammonium molybdate, and dissected for nerve-muscle relations. The central portions of the Primary and Secondary Longitudinal Tergals together with practically all of the Primary and Secondary Longitudinal Sternals have been removed in order to expose the nerves and more peripheral muscles. For clearness the three main nerve branches on each side of the ganglion have been slightly separated in dissection. All muscles seem in normal condition. Nerves running beneath muscles are shown in dotted lines, but no attempt has been made to show the complete system of nerve branches.


nc
pls
tls
lpsp
portion of sls
slt
plt
dva
qnlt
N,s
N1
N2
dvm
N,m
tlt
qlt
N3
sxlt
dvp
qls
tls
dvo
rmn

9

POTENTIAL DIFFERENCES ACROSS THE CHORION OF THE *FUNDULUS* EGG.

MARGARET SUMWALT.*

(From the Department of Physiology, Medical School, University of Pennsylvania, Philadelphia, and the Marine Biological Laboratory, Woods Hole.)

Loeb and Cattell (16) in 1915 reported the results of certain experiments on *Fundulus* eggs, which they did not satisfactorily explain. The central observation was that when KCl had penetrated the egg in sufficient amount to stop the heart of the embryo, it could escape, effecting recovery of the heart beat, if the eggs were placed in a solution of some other salt or in a dilute solution of acid, but not if they were placed in distilled water. An analogous observation on very different material was made ten years later by Michaelis and Fujita (20, 21), who found that K and other cations will pass through apple skin and dried collodion membranes into salt solution, but not into distilled water. Their explanation was that these membranes are permeable for cations but not for anions; when it is possible for cations on the two sides of such a membrane to be exchanged, movement of cations across the membrane can occur, but not otherwise. This explanation Michaelis, with several collaborators, has supported by abundant electrical and chemical evidence (5, 20–26).

In the present experiments some of the electrical methods used by Michaelis and his collaborators in the study of apple skin and artificial membranes have been applied to the chorion of the *Fundulus* egg. The results obtained indicate that the electrical properties of this membrane are similar in many respects to those of dried collodion membranes. If they are interpreted analogously, a partial explanation of the results of Loeb and Cattell is afforded.

* I wish to express my indebtedness and gratitude to Dr. M. H. Jacobs who suggested this application of electrical methods to the study of the permeability of the *Fundulus* egg, and to Dr. William R. Amberson with whom the problem was at first prosecuted jointly. Part of the work was done during my tenure of a Dean Van Meter Alumnæ Fellowship from Goucher College.

Material and Method.

The *Fundulus* embryo is enclosed by two membranes: the chorion, a non-cellular, tough, but elastic shell, and the ectoderm of the embryo. In the experiments to be reported, potential differences were measured across the chorion only. It is hoped, however, that a similar study of the ectoderm may be made in the near future.

The properties of the chorion do not appear to vary greatly with the age of the egg, except for a slight decrease in elasticity with time. Fertilized eggs of any convenient age could therefore be used. Most of those employed in these experiments were between 5 and 9 days old at a season when hatching occurred between the 10th and 13th day. A few experiments were performed with satisfactory results on unhatched cold storage eggs 28 days old.

Potential differences were measured between the inside and the outside of the chorion of single eggs. The subchorionic fluid surrounding the embryo constituted a constant environment for the inner surface of the membrane, while solutions of various compositions and concentrations were applied to the outside. In an ideal system, successive applications of two different solutions to the outside of a membrane should produce a change in the membrane potential equal to the P.D. which would be observed if the membrane were placed between the solutions in question. Though the egg is not an ideal system, potential differences arrived at by this method are probably not greatly in error. Measurements across apple skin are, of course, subject to the same disadvantage, but in the hands of Fujita (5) have, by the use of the method here employed, yielded significant results.

To make the inside electrical contact, a capillary pipette was inserted into an egg so that its orifice lay in the subchorionic fluid between the embryo and the chorion. The pipette was filled with saturated KCl and communicated with a saturated KCl calomel half cell. Outside contact was made by dipping a like half cell into the solution in which the egg was immersed. The arrangement of the apparatus is shown schematically in Fig. 1.

The two electrodes were connected into a simple potentiometer circuit. A Leeds and Northrup student potentiometer was used, and the null instrument was a d'Arsonval galvanometer of high

sensitivity. The electrical resistance of the system without an egg was from 10,000 to 100,000 ohms, according to the diameter of the pipette and concentration of the solution into which the electrodes dipped. With an egg on the pipette, the resistance was still higher. Since the galvanometer deflections diminished as the resistance increased, readings were more accurate when the egg lay in concentrated solutions than in dilute. The sensitivity of the galvanometer was sufficient to give a deflection of at least 1 mm. for 5 millivolts with an egg on the pipette in M/20,000 KCl. In this, the most dilute solution used in any of the experiments, the P.D. was sufficiently large to render the experimental error reasonably small (about 5 per cent.). In M/2,000 KCl the galvanometer deflections were of the order of 1 mm. for 1 millivolt, so that considerable accuracy was possible in the determinations at this and greater concentrations.

So high was the resistance in the circuit when an egg was impaled on the electrode that, because of the humid weather conditions prevailing at Woods Hole, and the presence in the laboratories of traces of salts from the sea water, none of the precautions originally used to shield the apparatus prevented short circuit leaks. The work must have been abandoned had not independence of weather conditions been finally secured in a dry room.[1] Here no leaks occurred so long as door and windows were kept closed.

When Osterhout, Damon, and Jacques (28) measured P.D. in *Valonia*, they immersed the cells only partly in the experimental solution, and tested for short circuits at the hole where the pipette entered the cell by comparing the values for part immersion with others obtained when the cell was completely submerged in the same experimental solution. The presence of a leak was shown by diminished P.D. in the latter case.

The *Pundulus* egg, however, is too small for partial immersion without danger of complete wetting by capillarity. In the present study, therefore, an egg was completely immersed in the experimental solutions throughout a determination. That little or no leakage occurs ordinarily under these conditions is indicated by

[1] This was a room in which there was no running water, which had never been used for any experimentation, and which had been closely shut up. Solutions were prepared elsewhere, and the area of free water surfaces was reduced to a minimum.

the constancy and reproducibility of the P.D.'s obtained. After puncturing an egg in sea water, successive washings in the first experimental solution usually gave steadily ascending P.D. values until a definite maximum was obtained, and this maximum was reproducible within certain limits which will be mentioned later. But, occasionally, the wound failed to close tightly around an entering pipette, and in such a case the observed P.D.'s were small and erratic. This behavior occurred with large pipettes and with pipettes improperly shaped for making a clean puncture, and was more frequent with older eggs in which the chorion was less elastic. Failure to obtain a tight seal about the electrode could often be detected by the visible escape of subchorionic fluid. But the presence of even an invisible leak was recognizable by the inconstancy of the observed P.D. Results on leaky eggs were always discarded.

The difference of potential between the electrodes alone, dipping directly into an experimental solution, amounted at times to 2 or 3 millivolts, but it was reproducible no matter what the dilution or composition of the solution, so long as sufficient pressure was maintained to keep a gentle stream of KCl issuing from the mouth of the pipette electrode. The density of the saturated KCl made this flowing junction visible. If the pressure dropped to zero, however, so that the visible flow of KCl ceased, anomalous P.D.'s were observed whose magnitude increased with the dilution of the solution surrounding the electrode tips. In the most dilute solutions used, these sometimes attained a magnitude of 100 millivolts or more. The site of these P.D.'s was the mouth of the pipette electrode, as was shown by short circuiting[2] it; and the cause, at least in part, its small size. Pairs of large tubes showed no such effect. Agar-filled tips of unequal size showed them even more markedly. All of the data presented in this paper have been

[2] The pipette electrode was short circuited as follows: As Fig. 1 shows, the pipette is not the only avenue of contact with its calomel half cell. There is also a communication with that half cell through a siphon dipping into a reservoir. When the P.D. between the calomel half cells was to be measured, free from the influence of the P.D. occurring at the mouth of the pipette, the experimental solution was placed in some vessel other than the egg chamber. Into this dipped the calomel half cell which usually made contact with the solution in which an egg was immersed; and the other half cell was put in contact with it through the siphon.

corrected for the electrode potential measured just before or just after each egg determination, with the electrodes dipping into sea water and a flowing junction at the mouth of the pipette electrode.

Although a flowing junction could be maintained between the pipette and the experimental solution during preliminary tests of the electrodes, such a junction was impossible between the pipette and the subchorionic fluid of an egg. In fact, to prevent contamination of the egg contents with saturated KCl, a pressure was maintained in the capillary which, though sufficient to produce a flowing junction in open solution, permitted a slight ascent of egg substance into the capillary when balanced against the turgor of the egg. Two considerations, however, support the belief that the experimental data are free from artefacts produced by the electrodes. First, the tests of the electrodes alone show that high anomalous P.D. values were due to the pipette and appeared only when it was in dilute solutions. During a measurement of P.D. across the chorion the pipette was in subchorionic fluid, the concentration of which was of the same order as that of sea water. The pipette was thus protected by the egg against the environment in which the high P.D. at its mouth was produced. Second, the subchorionic fluid—KCl junction in the pipette was constant throughout an experiment. If there was a P.D. at this junction, the effect which it had disappears when *differences* between observed P.D.'s are considered; and this is the case with all the results given.

Pressure control in the pipette electrode was desirable for the two reasons already discussed: to maintain a flowing junction during the preliminary tests of the electrodes; and to prevent a flowing junction during egg measurements. Therefore an apparatus patterned after that used by Landis (7) for capillary injections was used. (See Fig. 1.) A Luer syringe communicating with the pipette half cell system made small sudden changes of pressure possible, and a reservoir in communication with the system through a siphon at another point maintained a constant head of pressure, the influence of which could be controlled by a stop cock.

The pipette communicated with the pressure control through a coil of hard rubber tubing sufficiently flexible to permit control

of the movement of the pipette with a Chambers micromanipulator. The egg lay in a chamber on the stage of a microscope, and the position of the pipette within it could be observed at all times during the course of the experiments.

The inside diameters of the capillary electrodes used were about 70 μ. In experiments with eggs, no systematic variation in P.D. was observed with pipettes of different sizes, except when so large a one was used that the chorion failed to close tightly around it. The puncture of an egg was always carried out in sea water in order to avoid carrying into it excess KCl on the outside of the pipette.

As a precaution against contamination of the experimental solution by diffusion of saturated KCl from the outside electrode, the electrode dipped not directly into the egg chamber, but into a thistle tube communicating with the egg chamber through 7 or

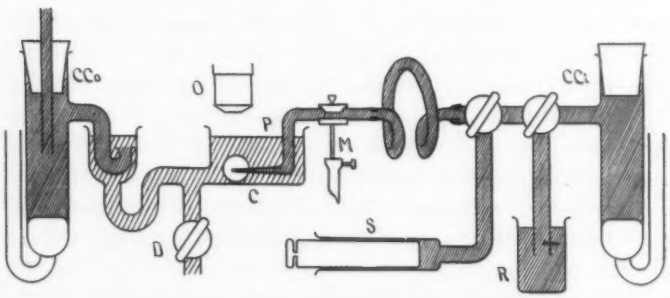


FIG. 1. Diagram of apparatus. The egg, impaled on the capillary pipette *P*, lay in a chamber *C* beneath the microscope objective *O*. Outside electrical contact was made through a calomel half cell *CCo* which dipped into the solution in which the egg was immersed at some distance from the egg, and drainage of the chamber was effected through the tube *D* at an intermediate point. The movements of the pipette were controlled by a Chambers micromanipulator *M*. The pipette is in communication through a coil of hard rubber tubing not only with the other calomel half cell *CCi*, but also with a Luer syringe *S* for pressure control, and with a reservoir *R*, the height of which was adjustable.

8 cm. of glass and rubber tubing (Fig. 1). Drainage of the chamber and the thistle tube was effected through a side arm midway between them. The solution was added by pouring it into the egg chamber from above. The outside electrode, itself, after

dipping into experimental solutions, could be flushed from a reservoir of saturated KCl and calomel.

It was found that variation in the magnitude of concentration potentials among different batches of eggs occurred, a striking fact in view of the negligible variation with age in the eggs from one female. A comparison of Tables 2, 3, 4, 5, and 8 shows, in KCl solutions at the same concentration range, slightly more than 100 per cent. variation of concentration potentials. Since conclusions drawn from these experiments are in every case based on relative rather than absolute values, however, the conclusions are not vitiated by this variation, because control experiments in any given study were always run within 24 hours on the same batch of eggs.

More difficult to cope with was the variation of P.D. across the same egg membrane, with time, and with successive washings in the same solution. This variation seemed to depend chiefly on differences in the thoroughness of washing; and secondarily on movements of the embryo within the egg, which disturbed the tightness of the electrical seal where the pipette penetrated the chorion. Probably in the brief time occupied by most of the experiments (less than half an hour for each egg), the factor of chorion permeability, which is discussed in connection with the experimental results of Table 1, was not important. The egg was washed with fresh solution after each reading until two successive readings were obtained which checked within about 5 per cent. A different experimental solution was then used.

The pH determinations required by the experiments were made with a quinhydrone electrode calibrated against standard buffer mixtures. The so-called neutral solutions were solutions of the pure salts made up in distilled water without the addition of any acid. Determinations of the pH of such solutions are of doubtful value, but a few which were made seemed to show that these solutions had a pH in the neighborhood of 5.4.

The experiments were carried out at temperatures which ranged from 20° to 26°, though they did not vary over more than 3° in the course of any one set of observations.

Experiments and Discussion.

One may recognize a membrane which is not equally permeable for all ions by the magnitude and direction of the potential differences to which it gives rise under certain sets of conditions. The dried collodion membrane has been shown by Michaelis and his colleagues (19, 22–27) to be of this sort. Out of the variety of criteria which these investigators have used to demonstrate the differential permeability of this and other membranes to ions, two were chosen for use with the *Fundulus* chorion. The first was the application to the membrane in question of two different concentrations of the same electrolyte solution; the second, the application of like concentrations of two different electrolytes.

When a dried collodion membrane (which is permeable for cations, but hardly, if at all, for anions) separates two different concentrations of the same electrolyte solution, a concentration potential results of such polarity that the dilute solution is positive with respect to the more concentrated solution. For any given electrolyte two features of such a concentration potential are of interest, namely, the sign, which is dependent on the greater permeability for cations, and the magnitude, which is a function of the degree of difference between the cation and the anion permeability. In the best dried collodion membranes the magnitude of the concentration potential with an electrolyte of univalent ions is close to the maximum theoretically possible (19) with a membrane perfectly impermeable for anions, but permeable for cations.

When a single egg was exposed to a series of dilutions of a KCl solution ranging by tenfold steps from M/2 to M/20,000, a series of concentration potentials was obtained (Table 1). In M/2 KCl (which is approximately isomotic with sea water) the outside solution was usually slightly negative with respect to the contents of the egg. All the more dilute solutions were positive with respect to the egg. The more dilute the solution, the greater was the degree of this positivity. The sign of these concentration potentials, therefore, is indicative that the chorion is more permeable for cations than for anions. The direction of polarity in M/2 KCl is such as would be obtained if the subchorionic fluid of the egg were slightly less concentrated with respect to electrolytes than this solution, though more concentrated than the rest.

The magnitude of the negative P.D.'s, however, is too small for much emphasis to be laid upon this point.

TABLE I.

CONCENTRATION SERIES.

Potential differences between the inside and the outside of 5 eggs immersed in a succession of KCl solutions of the indicated concentrations. The sign of the outside solution was positive except where the negative sign occurs.

No.	Age in Days.	$\frac{M}{2}$.	$\frac{M}{20}$.	$\frac{M}{200}$.	$\frac{M}{2,000}$.	$\frac{M}{20,000}$.	$\frac{M}{2,000}$.	$\frac{M}{200}$.	$\frac{M}{20}$.	$\frac{M}{2}$.
1	5	0.8 −	0.3	10.6	55.9	107.7	46.7	3.7	0.8 −	0.8 −
2	7	2.4	5.3	18.0	55.0	99.2	56.0	8.8	0.7	0.4
3	7	0.3 −	3.1	14.5	49.7	95.7	58.0	17.8	1.2	0.3
4	7	0.1 −	0.7	6.7	37.9	91.9	53.6	8.2	0.6	0.3 −
5	6	1.9 −	1.6	13.1	44.8	84.3	50.9	10.7	2.4 −	1.9 −

It will be observed in Table 1 that the concentration potentials (*i.e.*, the changes of P.D. between any two successive readings) increase markedly with dilution. Those from the ascending part of the series are shown in Table 2. The average value rises from

TABLE II.

VARIATION OF CONCENTRATION POTENTIALS WITH DILUTION.

Concentration potentials obtained by subtracting adjacent values in the ascending parts of the series shown in Table I. The more dilute solution was positive.

No.	$\frac{M}{2} - \frac{M}{20}$.	$\frac{M}{20} - \frac{M}{200}$.	$\frac{M}{200} - \frac{M}{2,000}$.	$\frac{M}{2,000} - \frac{M}{20,000}$.
1	1.1	10.3	45.3	51.8
2	2.9	12.7	37.0	44.2
3	3.4	11.4	35.2	46.0
4	0.8	6.0	31.2	54.0
5	3.5	11.5	31.7	39.5
Average	2.3	10.4	36.1	47.1

one negligibly small in the interval between M/2 and M/20 to 47.1 mv. between M/2,000 and M/20,000, which is of the order of magnitude of the maximum of 58 mv. theoretically possible for a membrane completely impermeable for anions. In this be-

havior the egg membrane resembles a "poor" or large pored dried collodion membrane (24, 25). "Good" dried collodion membranes give very nearly the theoretical maximum even in fairly concentrated solutions.

An important feature of such a concentration series is the reversibility and reproducibility of the P.D.'s obtained when an egg is exposed to the same series of concentrations in the reverse order. In the experiments summarized in Table 1 each reading was obtained after the egg had been washed in at least six changes of the solution, but more prolonged washing, until two readings were obtained that checked, was not attempted. Such a series required for its completion approximately 45 minutes, with an exposure of about 5 minutes to each solution. The values obtained in this way were, therefore, probably lower on the ascent, and higher on the descent, than the definitive values for the concentrations in question; but the similarity shown by the experimental results for ascent and descent makes it appear probable that they were actually close approximations to these definitive values. The fact that in most cases the descending value was higher than the ascending one in the same solution indicates that dilution of the subchorionic fluid did not occur during the experiment, since this would have diminished the second P.D. observed.

When the concentration potentials obtained across a membrane with solutions containing ions of different valence are compared, further information is furnished as to the differential permeability of the membrane. If it is impermeable for anions but permeable for cations, concentration potentials across it are independent of the valence of the anion in the electrolyte solutions used, but are, theoretically, halved by doubling the valence of the cation (19).

The concentrations M/200–M/2,000[3] were arbitrarily chosen as a test range for the study of concentration potentials with salts yielding bivalent anions or cations. It was found that the concentration potential for a bivalent anion, SO_4, was identical with that for the univalent Cl. Table 3 shows typical results on 5 eggs in KCl and 5 others in K_2SO_4. There was less than 1 mv. of difference between the averages. This failure of the valence of

[3] The concentrated solution was always used before the dilute.

the anion to affect the concentration potential is another point of resemblance between the *Fundulus* chorion and the dried collodion membrane, and is additional evidence that the chorion is at least relatively impermeable for anions.

TABLE III.

EFFECT OF ANION VALENCE ON CONCENTRATION POTENTIALS.

Concentration potentials were measured between M/200 and M/2,000 solutions of KCl and K_2SO_4, a different egg being used for each measurement. The more dilute solution was positive.

KCl.	K_2SO_4.
32.4	39.3
32.7	33.5
33.7	29.3
30.3	33.6
38.4	36.2
Average 33.5	34.4

On the other hand, when it was the cation whose valence was doubled, the concentration potential across the *Fundulus* chorion was approximately halved. The effect was shown with $CaCl_2$, $MgCl_2$, and $BaCl_2$ (Table 4). The behavior of the chorion is

TABLE IV.

EFFECT OF CATION VALENCE ON CONCENTRATION POTENTIALS.

Concentration potentials were measured between M/200 and M/2,000 solutions of KCl, $CaCl_2$, $BaCl_2$, and $MgCl_2$. The effects of Ba and Mg were not studied in the same batch of eggs with Ca, and are therefore exhibited with a different set of controls in KCl. In every case the more dilute solution was positive.

	KCl.	$CaCl_2$.	KCl.	$BaCl_2$.	$MgCl_2$.
	39.0	17.1	18.8	6.4	9.9
	31.6	16.3	23.7	15.4	13.0
	29.4	14.5	21.3	9.7	6.8
	30.9	13.8	22.7	9.8	14.1
	33.5	17.0	19.5	6.6	11.9
Average . .	31.6	15.7	21.2	9.6	11.1

precisely what would be predicted by the simplest theory for an ideal membrane. It is different, however, from the behavior of a

dried collodion membrane, which with $CaCl_2$ gives no concentration potential at all, because it happens to be impermeable for Ca as well as for Cl (30).

Most of the experiments with valence effects of cations were made with equimolecular solutions of the different salts, *i.e.*, solutions containing equal numbers of cations. In a few experiments equivalent solutions, containing equal numbers of anions were used for comparison. Thus, as an example of the latter type of experiment, the concentration potential for M/400–M/4,000 $CaCl_2$ instead of M/200–M/2,000 $CaCl_2$ was compared with that for M/200–M/2,000 KCl. As might have been expected, the results obtained in this way (Table 5) did not differ greatly from those in which equimolecular solutions were employed.

TABLE V.

EFFECT OF CATION VALENCE ON CONCENTRATION POTENTIALS. CHOICE OF CONCENTRATION RANGE.

Concentration potentials with KCl and $CaCl_2$ were compared in both equivalent and equimolecular solutions. Two sets of controls in KCl appear because the $CaCl_2$ experiments were made on different days.

	KCl, $\frac{M}{200} - \frac{M}{2,000}$.	$CaCl_2$, $\frac{M}{400} - \frac{M}{4,000}$.	KCl, $\frac{M}{200} - \frac{M}{2,000}$.	$CaCl_2$, $\frac{M}{200} - \frac{M}{2,000}$.
	31.5	20.7	32.7	14.2
	42.5	19.4	44.2	18.4
	23.6	9.4	40.3	11.7
Average...	32.5	16.5	39.1	14.8

Still further evidence for the relative impermeability of the chorion for anions was obtained when the second test was applied: *i.e.*, the exposure of the egg to a series of salt solutions alike in concentration but differing in composition. Against the dried collodion membrane equal concentrations of different salt solutions containing the same cation are isoelectric; but when the anion is the common ion, they give rise to P.D.'s differing in magnitude in the same order as the classic mobility values of the cations used (19).

Tables 6 and 7 show the P.D. in mv. between the inside and outside of eggs each of which was exposed successively to all the

solutions shown in that table. The values obtained with different eggs in the same solution are of no interest, except to show the fair constancy of the material; but the values obtained with a

TABLE VI.

P.D. AGAINST DIFFERENT ANIONS OF THE SAME CONCENTRATION.

P.D.'s between the solution and the egg contents are given from six typical experiments in each of which one egg was exposed to all of the following salts of K. Variation of the order in which the solutions were used had no effect.

No.	Cl.	Br.	I.	SCN.	Acetate.	NO_3.
1	46.4	40.8	44.0	44.1	42.1	44.2
2	48.6	43.9	49.2	47.5	44.6	45.5
3	50.6	45.6	50.9	48.8	44.0	46.9
4	52.6	45.6	49.7	50.0	50.8	45.5
5	41.7	42.1	43.5	43.2	41.0	41.5
6	42.1	42.4	42.0	40.0	37.9	40.9
Average	47.0	43.4	46.5	45.6	43.4	44.1

given egg in different solutions are of interest, and the averages of these indicate the general trend of the effects. It will be seen from Table 6 that there is no difference of potential which may be

TABLE VII.

P.D. AGAINST DIFFERENT CATIONS OF THE SAME CONCENTRATION.

Single eggs were exposed to a succession of M/2,000 solutions of the following chlorides. P.D.'s across the chorion from 5 typical experiments are given. Variation of the order in which the solutions were used had no effect.

No.	Li.	Na.	K.	Rb.	Cs.
1	61.3	55.0	54.0	38.1	31.9
2	47.8	48.6	48.7	28.7	29.8
3	59.3	49.3	48.1	33.4	32.1
4	58.8	58.4	49.2	37.7	31.7
5	63.4	57.9	56.1	37.8	38.2
Average	58.1	53.8	51.2	35.1	32.7

considered significant among the K salts of Cl, Br, I, SCN, acetate, and NO_3. But when the anion is kept constant and the cation is varied, a series appears in which the cations, Li, Na, K,

Rb, and Cs, are arranged in the order of their classic mobility values, LiCl being positive to all the other chlorides used, and CsCl negative. It is interesting to note that while the order for the series is correct, the values obtained with Li, Na, and K are very close together, while those with Rb and Cs fall in a separate group at some distance from the others; whereas the most pronounced break in the mobility values for free diffusion is not between K and Rb, but between Na and K. The results of this experiment may be interpreted to mean not only that the membrane possesses differential permeability for ions of opposite sign, but also that differences are present, though to a lesser degree, in the permeability for univalent cations. Of the ions studied, Cs appears to penetrate most readily, and Li least readily.

The results of these experiments, in which the dilution, valence, and chemical identity of the different ions in the solution applied to the membrane has been systematically varied, may be confidently interpreted to mean that in approximately neutral solutions the chorion is more permeable for cations than for anions. This conclusion may be used as the basis of the following partial interpretation of Loeb and Cattell's results (16), though the complete explanation must be impossible until the electrical properties of the ectoderm have been studied in addition to those of the chorion.

If K, in order to stop the heart-beat of a *Fundulus* embryo, must penetrate both chorion and ectoderm, then recovery can be effected only by exit of K through that double membrane. The escaping K must either be accompanied by anions in an equivalent amount or exchanged for cations from the outside solution. Most of the movement of the cations must be accomplished in the second way because, as the present experiments indicate, anions pass with difficulty across the chorion. Therefore K escapes much more slowly into distilled water than into a solution, whether of salt or of acid.[4] The same explanation holds for the retardation of K penetration into eggs which have been soaked for 24 hours in distilled water. In these, Armstrong (2) has found that the sub-

[4] McClendon (17) gave this explanation briefly for the failure of Mg to escape from *Fundulus* eggs into distilled water; but when he reported later (18) that Mg also failed to escape into Van't Hoff's solution, he offered a different explanation, not only for this, but for the former result.

chorionic fluid has the pH of the surrounding medium. It seems probable, therefore, that the subchorionic fluid in these eggs has been largely replaced by distilled water.

The results of other experiments by Loeb are not so easily related to the the theory of differential permeability of the chorion for ions; for instance, the observation (11, 12, 13, 16) that K enters unwashed eggs more readily from a pure KCl solution than from a mixture of KCl with some other electrolyte. The additional electrolyte in this case may perhaps alter the degree of differential permeability of the membrane. This possibility will be mentioned in another connection.

Although measurements of P.D. yield direct evidence for the relative numbers of ions of opposite sign penetrating the membrane, the absolute numbers are not so directly indicated. Results of the type obtained would be possible under several states of ion permeability. For example, ions of both signs may traverse the chorion fairly readily, but at different rates; or both may fail almost completely to penetrate, though having sufficiently different penetrating tendencies to yield a P.D.; or, finally, cations may pass without anions. The results of several investigators working with different methods and criteria make it appear that the chorion is at least somewhat permeable for cations. Loeb (11, 19) found the eggs permeable for a dye cation, neutral red. Armstrong (3) showed that when heart standstill was brought about by excess K or acid in the surrounding solution there was no difference between the kind of effect on naked embryos and on embryos surrounded by a chorion. Bodine (4) has reported that the only difference under such circumstances is one of time. The chorion is probably permeable for all ions applied in sufficiently concentrated solutions or over long enough periods of time; and for cations even in dilute solutions, provided that electrical neutrality can be maintained by an exchange with other cations.

Several studies by Loeb (8, 9, 10, 14, 15) and one by Armstrong (3) on salt antagonism for acid penetration into *Fundulus* eggs, as well as a few experiments reported by Loeb (10) and Loeb and Cattell (16) on the opposite, namely, acid antagonism for salt penetration, have been of considerable interest; yet their

mechanism is imperfectly understood. It was thought, therefore, that an investigation of potential differences across the membranes of eggs in acid solutions might throw some light on this problem. Accordingly, the first of the tests used on eggs in neutral solutions was made, *i.e.*, the application to the chorion of different concentrations of the same electrolyte solution, with the modification that the solutions were brought to a desired pH value by the addition of an appropriate acid. The sign and magnitude of the concentration potentials were studied, and a comparison was made of the effects on them of di- and uni-valent ions.

When an egg was exposed in succession to two solutions of KCl, one M/20, the other M/200, to both of which sufficient HCl had been added to bring the pH to 3.0, a concentration potential was obtained with the polarity the reverse of that found in neutral solutions; that is, the more dilute solution was negative to the more concentrated. The experimental results given in the middle column of Table 8 deal with 5 eggs, each of which was studied in

TABLE VIII.

REVERSAL OF KCl CONCENTRATION POTENTIALS WITH INCREASE IN THE H ION CONCENTRATION.

Concentration potentials were measured between M/20 and M/200 solutions of KCl to which sufficient HCl had been added to give the desired pH. A different egg was used for each measurement. Sign of the dilute solution was positive except where the negative sign occurs.

pH.				
2.0	2.5	3.0	3.5	4.0
0.5	1.7 −	5.8 −	4.7 −	38.2
1.3	9.1 −	8.9 −	3.2 −	34.0
0.7	7.4 −	9.6 −	18.0	46.7
2.1 −	9.8 −	10.0 −	3.4 −	30.6
4.4	11.8 −	12.6 −	9.8	37.7

CONTROLS.

Concentration potentials previously shown by the same 25 eggs between M/20 and M/200 solutions of pure KCl.

18.4	23.1	20.9	25.6	35.3
25.8	26.5	22.0	28.4	38.0
22.6	25.4	——	30.9	39.7
21.1	24.3	——	30.8	40.4
16.3	25.7	25.5	33.0	36.8

pure solutions of KCl as well as in KCl at pH 3.0. The concentration potential values obtained at pH 3.0, while smaller than those in pure KCl, were manifestly in the opposite direction.

The range of salt concentrations chosen for these experiments with acid solutions was higher than in the experiments with neutral solutions because it was desired to study the concentration effect of the salt itself, rather than that of total electrolyte content. The complexity introduced by the use of a solution containing two electrolytes is simplified somewhat if the salt is relatively concentrated as compared with the acid. For the same reason, a pH of 3.0 was chosen for the subsequent comparison of KCl with other salts, rather than one of 2.0 or 2.5, where the acid would be a more significant element in the concentration. The addition of HCl in equal amounts to both concentrated and dilute solutions reduces the ratio of their concentrations with respect to total electrolyte. At a pH of 3.0 the HCl has approximately a concentration of 0.001 M. Thus the total electrolyte concentrations of the two solutions compared were 0.051 M and 0.006 M, respectively, and their ratio was 8.5 instead of 10. At pH, 2.5, their ratio was approximately 6.6, and at pH, 2.0, 4. The order of these relationships is not altered if activities are substituted for concentrations. The concentration potentials to be expected in acid solutions must therefore be less, in accordance with this reduction of the concentration ratios.

Despite the disadvantages of the more acid solutions (both because of their disturbance of the concentration ratio, and also because of their destructive effect on the membrane, to be referred to later), a study of concentration potentials was made at a series of different pH values in order to determine, if possible, the point at which reversal occurs. Table 8 also shows the results of these experiments, presenting under each of five pH values the concentration potentials obtained in both acid and neutral solutions with the same eggs. At a pH of 4.0, the concentration potentials were found to be in the same direction as the control values, and very slightly smaller. At 3.5, all were much reduced and 3 out of 5 were reversed. At 3.0 and 2.5 all were reversed and of considerable magnitude, but at 2.0 there was scarcely any concentration potential in either direction. Apparently the reversal point

lies slightly above a pH of 3.5, probably in the neighborhood of 3.7.

The marked reduction of the concentration potentials at pH 2.0 was in part to be expected because of the reduction of the concentration ratio which the addition of acid brings about. But it seems probable that at this lower extreme of the pH range there is also a destructive effect of the acid on the membrane which occurs too rapidly to permit detection of the characteristic potential differences. In harmony with this suggestion are two instances, shown in the column for pH, 3.0 in which, contrary to the procedure with the other eggs, the acid solutions were used before the neutral ones; with the result that no concentration potential was obtained in the subsequent control experiment. Apparently even this dilution of acid, in a period of 10 or 15 minutes, exercised some irreversible destructive effect on the membrane which abolished its differential permeability for ions.

The acid reversal of the sign of concentration potentials in KCl suggested the possibility that acidity might operate also to reverse the valence effect on concentration potentials, so that $CaCl_2$ would give values equal to those obtained with KCl, and K_2SO_4 values less by half. To test this theory, 5 eggs were studied in $CaCl_2$ solutions M/20 and M/200 at pH 3.0, and 5 other eggs in equimolecular solutions of KCl at the same reaction. It was found (Table 9), as had been expected, that concentration potentials with

TABLE IX.

EFFECT OF CATION VALENCE ON CONCENTRATION POTENTIALS AT PH 3.0.

Concentration potentials were measured between M/20 and M/200 solutions of KCl and $CaCl_2$, all brought to a pH of 3.0 with HCl. A different egg was used for each measurement. Sign is that of the dilute solution.

KCl.	$CaCl_2$.
14.5 —	23.3 —
13.1 —	13.8 —
13.4 —	19.2 —
7.6 —	20.6 —
4.4 —	19.0 —
Average 10.6 —	19.2 —

$CaCl_2$ at this pH were also reversed and were not smaller than those obtained with KCl. Indeed they were considerably larger.

(The difference was somewhat greater when they were compared in equivalent concentrations.) On the other hand, K_2SO_4, gave concentration potentials of the same sign as at neutrality (Table 10). The reversal point with K_2SO_4, if one exists, must be at a pH lower than 3.0.

TABLE X.

EFFECT OF ANION VALENCE ON CONCENTRATION POTENTIALS AT PH 3.0.

Concentration potentials were measured between M/20 and M/200 solutions of KCl and K_2SO_4 adjusted to a pH of 3.0 with HCl and H_2SO_4 respectively. A different egg was used for each measurement. The dilute solution was positive except where the negative sign occurs.

KCl.	K_2SO_4.
11.2 —	22.3
10.2 —	13.6
4.4 —	28.8
12.1 —	15.0
16.6 —	8.9
Average 10.9 —	17.7

Such results as these acid effects on concentration potentials are not obtained across dried collodion membranes (19). An inversion of concentration potentials with increased acidity has been reported for membranes of other materials by Mond (27), Fujita (6), Rein (29), and Amberson and Klein (1), but not for any membranes across which chemically controlled diffusion experiments have also been made. Nevertheless, logically interpreted, the reversal of concentration potentials across the *Fundulus* chorion in KCl and $CaCl_2$ solutions seems to mean that in acid solutions of those salts the chorion is more permeable for anions than for cations. The reversal point, then, is the pH where the membrane is equally permeable for ions of both signs.

The application of these results to interpretation of the studies by Loeb and by Armstrong of the antagonistic action between salt and acid is very difficult. More electrical experiments are needed, testing the effect of a greater variety of salts, concentrations, and pH values; but the direction in which such further experiments will be useful may be indicated here. We may assume that the hindrance either of KCl or of acid penetration involves a decrease in the permeability of the membrane for cations. But, as has already been pointed out, the actual number of ions

of either sign which penetrates cannot be directly determined from measurements of P.D. Equal ion permeability at the reversal point may be due to a diminution in permeability for cations, or to an increase in that for anions, or to both. So far, then, as acid antagonism for salt penetration may be correlated with the present results, Loeb's experiments (10) give more than they receive of illumination: because the fact of acid antagonism for salt penetration implies that the reversal point in the electrical experiments is produced mainly in the first way, *i.e.*, by diminution in permeability for cations.

At least two features of the results secured have not been explained. These are the facts that concentration potentials with $CaCl_2$ exceed those with KCl at pH 3.0, and that concentration potentials with K_2SO_4 are not reversed at all at that reaction. These facts seem to mean that the properties of the chorion are not due entirely to the pH of the medium, but depend also on its salt content. They may perhaps furnish a clue to the way in which salt antagonism for acid penetration, and perhaps also for the penetration of other salts, may be brought about. However, it should be remembered that the simple interpretation of the potential differences obtained across the chorion in terms of its differential permeability for ions does not explain the more fundamental question of how such differences in its ion permeability are produced.

Summary.

1. Potential differences were measured across the chorion of single eggs of *Fundulus heteroclitus*. The chorion was shown by three lines of evidence to be more permeable for cations than for anions:

a. Concentration potentials were of such a sign that the dilute solution was positive to the concentrated.

b. Concentration potentials with K salts of divalent and univalent anions were equal, whereas concentration potentials with chlorides of divalent cations were about half of those with K.

c. Equal concentrations of different anions were eqipotential against the egg, whereas equal concentrations of different cations gave various potential differences whose magnitudes were in the

same order inverted as the mobilities of those cations in free diffusion.

2. The difference between the permeability of the chorion for anions and its permeability for cations increased with dilution of the solution in which the egg was immersed.

3. In KCl and $CaCl_2$ solutions the ratio of chorion permeability for anions to permeability for cations increased with the H ion concentration, and was inverted with sufficiently increased acidity.

4. The pH of the reversal point, where permeability for anions was equal to permeability for cations, depended on the salt used. For KCl in the concentrations used it lay in the neighborhood of 3.7.

BIBLIOGRAPHY.

1. **Amberson, W. R., and Klein, H.,**
'28 J. Gen. Physiol., XI., 823.
2. **Armstrong, P. B.**
'27 Proc. Soc. Exp. Biol. and Med., XXV., 146.
3. **Armstrong, P. B.**
'28 J. Gen. Physiol., XI., 515.
4. **Bodine, J. H.**
'28 Biol. Bull., LIX., 396.
5. **Fujita, A.**
'25 Biochem. Z., CLVIII., 11.
6. **Fujita, A.**
'25 Biochem. Z., CLXII., 245.
7. **Landis, E. M.**
'26 Am. J. Physiol., LXXV., 548.
8. **Loeb, J.**
'12 Biochem. Z., XLVII., 127.
9. **Loeb, J.**
'12 Science, XXXVI., 637.
10. **Loeb, J.**
'15 J. Biol. Chem., XXIII., 139.
11. **Loeb, J.**
'15 Proc. Nat. Acad. Sc., I., 473.
12. **Loeb, J.**
'16 Proc. Nat. Acad. Sc., II., 511.
13. **Loeb, J.**
'16 Science, XLIV., 574.
14. **Loeb, J.**
'17 J. Biol. Chem., XXXII., 147.
15. **Loeb, J.**
'22 J. Gen. Physiol., V., 231.
16. **Loeb, J., and Cattell, McK.**
'15 J. Biol. Chem., XXIII., 41.

17. **McClendon, J. F.**
'11 Am. J. Physiol., XXIX., 289.
18. **McClendon, J. F.**
'13 Science, XXXVIII., 280.
19. **Michaelis, L.**
'25 J. Gen. Physiol., VIII., 33.
20. **Michaelis, L., and Fujita, A.**
'25 Biochem. Z., CLVIII., 28.
21. **Michaelis, L., and Fujita, A.**
'25 Biochem. Z., CLXI., 47.
22. **Michaelis, L., and Fujita, A.**
'25 Biochem. Z., CLXIV., 23.
23. **Michaelis, L., and Hayashi, K.**
'26 Biochem. Z., CLXXIII., 411.
24. **Michaelis, L., and Perlzweig, W. A.**
'27 J. Gen. Physiol., X., 575.
25. **Michaelis, L., Ellsworth, R. McL., and Weech, A. A.**
'27 J. Gen. Physiol., X., 671.
26. **Michaelis, L., Weech, A. A., and Yamatori, A.**
'27 J. Gen. Physiol., X., 685.
27. **Mond, R.**
'24 Arch. ges. Physiol., CCIII., 247.
28. **Osterhout, W. J. V., Damon, E. B., and Jacques, A. G.**
'27 J. Gen. Physiol., XI., 193.
29. **Rein, H.**
'26 Z. Biol., LXXXV., 195.
30. **Weech, A. A., and Michaelis, L.**
'29 J. Gen. Physiol., XII., 487.

HISTORY OF THE DISCOVERY OF PERIODIC REVERSAL OF HEART-BEAT IN INSECTS.[1]

JOHN H. GEROULD,

DARTMOUTH COLLEGE, HANOVER, N. H.

Malpighi's discovery of periodic reversal of direction in the heart-beat of the silkworm pupa and moth has remained almost in oblivion for nearly 260 years. Réaumur held that in the pupa and adult moth the heart beats backward; in the caterpillar, forward. Cornalia confirmed Malpighi's observations regarding the pupa, but made no observations on the adult. Bataillon's description of periodic reversal during and around metamorphosis is mainly correct, but he wrongly held that inverse (backward) circulation exclusively occurs in the pupa after the first few hours and forward circulation exclusively in the adult. Fischer and Gerould independently discovered periodic reversal in the chrysalids of various Lepidoptera (*Colias*, etc.). Bataillon and Fischer regarded reversal and slow backward beating as due chiefly to increased acidity of the blood at metamorphosis. Gerould later found that periodic reversal is not transitory but characteristic of the adult in Lepidoptera generally, as well as of the prepupa and pupa, and that, as in Ascidians, it cannot be explained by the back-pressure hypothesis which he at first advanced. Its explanation must be sought in chemical conditions, and these conditions persist to the end of adult life.

The phenomenon of periodic reversal in the direction of peristalsis of the heart in Ascidians is well-known to zoölogists. The heart beats rapidly backward, in the direction of the viscera, a

[1] Introductory to a series of papers reporting investigations assisted by a grant from the Joseph Henry Fund. The major part of this paper was written at the Laboratoire d'Entomologie in Paris of Professor E. L. Bouvier, for whose friendly assistance, as well as for that of Drs. F. Le Cerf, L. Berland and others of the staff, the writer makes grateful acknowledgment.

certain number of times (15–83 in *Ascidia atra*, Hecht, 1918), and then, reversing, beats more slowly, a smaller number of times (14–43), forward and toward the branchial sac. Advisceral and abvisceral phases alternate with each other, the former being the longer and more vigorous.

It is not generally known, however, that a similar phenomenon occurs in mature Lepidoptera. The dorsal vessel or heart in the caterpillar beats forward, as in insects generally, but in the prepupa, pupa and adult alternating phases of rapid forward and slower backward pulsations occur. Forward phases in the moth or butterfly resemble in strength and rapidity of pulsation the advisceral or backward phases in the Ascidian. Backward phases in the moth resemble abvisceral or forward phases in the Ascidian in being as a rule less rapid. On the basis of observations on translucent pupæ of various butterflies and moths, I first reached the conclusion that the phenomenon is universal in the pupæ of Lepidoptera. The fact that it continues through adult life in the silk-worm moth, *Telea polyphemus, Actias luna, Samia cynthia, Sphinx chersis, Ctenucha virginica, Notolophus leucostigma, Argynnis aphrodite,* etc., leads me to think that the same is probably true of all adult Lepidoptera.

Whether a corresponding phenomena occurs in any other order of insects is still unknown. A statement by Kirby and Spence (1828) suggests that it probably has been observed in the drone fly, *Syrphus pyrastri* (see below, p. 224).

It is a curious fact that Malpighi's discovery (1669) of periodic reversal in direction of peristalsis in the heart of the silkworm has been buried almost in oblivion for nearly 260 years. His account dealt chiefly with the adult moth, about which his successors have had most diverse and conflicting opinions.

During the past two summers (1927, 1928) I have had the opportunity to study the phenomenon of periodic reversal of heartbeat in the silkworm from its beginning in the mature larva to the end of the life of the adult moth, and have found that Malpighi's brief description based on the vivisected moth was in the main correct. It was an extraordinary achievement when one considers the limitations of the lenses at his command.

Recognition of his discovery has been balked by various preju-

dices such as the idea prevalent in the early part of the nineteenth century that there is no circulation of hæmolymph in insects and by the equally false idea now widespread in scientific circles that the insect heart is always provided with valves between real or imaginary chambers so situated as to prevent the blood from flowing backward. Such chambers and such valves have no existence in the silkworm heart, which is a simple muscular tube. The valvular ostia are so small in the adult that they are difficult to detect even in stained and cleared preparations. They were carefully studied by means of sections by Verson ('08) and found to be lateral bottomless pockets extending forward from near the posterior part of each muscular *ala cordis,* the fan-shaped " wings " which extend laterally from the heart in intersegmental pairs. The ostia thus are not far from the middle of abdominal segments.

Examination of the beating heart of the silkworm moth makes it evident that the valves in the ostia have as little effect on the flow of blood backward within the tube as the abutments of a long bridge spanning a river have upon the flow of water which washes past them. They are so inconspicuous that Cornalia (1856) and Maestri ('56) did not find them, and even such excellent modern treatises on the silkworm as that of Vieil (1920, p. 119) state that the heart is without ostia or valves and that the blood enters it by endosmosis.

The wrong conception of the silkworm heart as a chain of separate chambers was held even by Malpighi, who regarded the dorsal vessel as a series of little " hearts " with a high degree of independence of one another, but his description of periodic reversal is, nevertheless, remarkably accurate.

Of periodic reversal, he says in brief: " The movement in the hearts established during the first days of the chrysalis stage still continues, directed from the front parts backward, and a succession of systoles propel the liquid. But the nature of the movement [in the moth, laid open] is by no means constant, so that even a slight cause may produce a change; nothing, perchance, could be more variable." He illustrates by saying: " I remember to have seen in the moth (" Papilione ") the movement of the heart forward from behind, which is uncommon;[2] then, after a

[2] On the contrary, forward phases are as frequent as backward.

short time, the movement changed its point of departure, directing itself from in front backward and so continued for a long time." [3]

Another example is worth quoting: "Likewise, in a moth, the heart began to beat from behind toward the head; meanwhile the canal of the heart was cut across; the posterior section then beat forward from behind but in the following way: the hindmost part beat rapidly, that contiguous to it less frequently, whereas the other [anterior] portion beat in the opposite direction."

"In certain other adult moths, in which the heart had been similarly cut, the two separate parts showed contractions directed at first toward the head, then toward the tail, and the liquid flowed out at each pulsation." In a silkworm about to pupate, normal forward peristalsis was observed until he cut the ventral body wall, whereupon "the direction of the movement changed, and 70 pulsations were counted which ran freely backward along the whole length of the hearts; but presently the movement began again from tail to head and finally, by manipulating with the finger nail the posterior hearts, beating began once more from in front backward." [4]

Malpighi extended his observations to the pupa of a moth popularly called "Pino." He describes a fresh chrysalis in which the heart-beat was first from the head backward to the posterior extremity: "The liquid was propelled thence to the middle of the body, then from the middle back to the same [posterior] extremity, like a ball thrown back and forth by players, and this play of nature continued for a while, until two opposite movements began from the middle forward and backward; and at last only one persisted, which went from head to tail."

These are perfectly conceivable variations of inverse circulation. First complete backward pulsation, then, probably, conflicting pulsations imperfectly seen, then double action from the 3–4 abdominal segment, finally complete backward pulsation.

[3] Translation made from Maillot's ('78) French version.

[4] Malpighi was correct in finding that the dorsal vessel beats after the moth is apparently dead, but he drew on his imagination in describing the various movements which then occur in the "long series of partial hearts which intercommunicate." "In one of these hearts, he says, three beats occur, in the next heart only one or two; indeed variations occur in the same partial heart."

Passing on nearly a half century to Réaumur (1732) we find in his great work on insects no original observations on circulation in the pupa, but, after referring to Malpighi's account of reversal in the pupa and moth of the silkworm and its individual variations, he says: "However, if one will take the trouble to observe the movement of the blood in the big vessel of a large number of adult moths, he will be convinced that the true course is from the front backward, whereas in the caterpillar it is from behind forward." ("Dans le papillon, la vraye route est des parties supérieures vers les inférieures, au lieu que dans la chenille elle est des parties inférieures vers les supérieures.")

Thus Réaumur did not describe periodic reversal but states positively that in the adult moth and in the pupa peristalsis is backward, whereas in the caterpillar it is forward.

Herold (1823) expressly repudiated Malpighi's description of periodic reversal as a manifest error, for which he accounted as follows: The shortening of the dorsal vessel at pupation to about half its former length would give the enclosed blood much less room, the vessel being closed at both ends according to his conception. Hence the blood of the pupa propelled forward from the large posterior end might rebound from the smaller anterior extremity and make a wavelike movement backward. This might give anyone observing for the first time the pulsation in a pupa which had just shed its larval skin the impression of periodic reversal. "But," he adds, "such an apparently double direction of movement of the dorsal vessel has never come to my attention."

Cornalia (1856) very definitely confirmed the observations of Malpighi. In describing circulation in the chrysalis of the silkworm, he calls attention to the interesting fact, already noticed by Malpighi, denied by many, and recently reconfirmed by Professor De Filippi: "This movement operates first in one direction then in the other; that is to say, the blood is carried by certain pulsations from the anal to the head regions, and then, by others, from the head to the anus; in this respect the circulation of the chrysalis is strangely different from that of the caterpillar." He adds that probably the same phenomenon occurs in the perfect insect but that the opacity of the skin renders observation difficult. It evidently did not occur to him to rub the scales off from the back,

or he would have observed that the skin is, on the contrary, very transparent.

Bataillon's ('93) more detailed account of periodic reversal in the silkworm agrees in most respects with my observations so far as it applies to the mature caterpillar just preceding pupation, to which period his observations were mainly confined and for which they are trustworthy, but he was wrong in stating that backward or inverse circulation occurs *exclusively* in the chrysalis after the first few hours after pupation and that normal or forward pulsation exclusively occurs in the adult.

His conclusions are as follows:

1. Appearance on the second day of spinning of an inverse circulation alternating at regular intervals with direct circulation.
2. Gradual predominance of this inverse circulation.
3. Rising of the curve of direct circulation toward the period of pupation.
4. Indifferent circulation [double action in both directions from the middle of the body] during the few hours which precede and follow pupation.

To these four conclusions we may in general subscribe but the two following, as has been indicated, were based on insufficient evidence.

5. Inverse circulation exclusively during pupal life.
6. Reappearance of normal circulation toward the head, exclusively, the day before eclosion of the adult.

To Bataillon periodic reversed was a transitory phenomenon, due to asphyxiation, comparable to disturbances in circulation accompanying metamorphosis in tadpoles, which he had previously observed. It was not a new and permanent type of circulation characteristic of pupal and adult life, as I have found it to be.

Vieil ('20) states quite correctly[5] that in the chrysalis of the silkworm, the rare, irregular pulsations of the dorsal vessel appear to originate in the third abdominal segment and to move from there forward and backward. Thus he finds only double action in the

[5] True of the fresh chrysalis immediately after pupation. Later, phases of backward pulsation through the whole dorsal vessel alternate with phases of forward beating.

chrysalis. In the spinning caterpillar, quoting Maillot, he describes a backward phase of slow beating (9 beats per minute) alternating with a forward phase of rapid pulsations (50 per minute).

Entirely without knowledge of the previous observations on the silkworm, Fischer ('18) and Gerould ('24*a*, '24*b*) independently rediscovered periodic reversal of peristaltic heart movements in the pupæ of various lepidoptera.

Fischer, in 1900, while examining chrysalids of *Charaxes jasius* at a high temperature (38° C.), saw the heart suddenly cease beating and then after a long pause beat backward, continuing afterwards to alternate in the direction of pulsation until the pupæ were returned to room temperature (18° C.). He satisfied himself that, thereupon, the direction of the peristalsis became "normal," that is, forward. Later, a moth, *Deilephila vespertilio*, responded in a similar manner to the stimulus of heat, and *Charaxes jasius* to the mechanical stimulus of blows with a small rod of wood. In the summer of 1917 he observed in the mature larva of *Colias hyale* and fresh pupæ of *Pararge mæra* that "antiperistalsis" occurred at room temperature (18°–21° C.) without recognizable changes in external or internal conditions. He was impressed by the remarkable slowness of the pulse in antiperistalsis, as well as by the variability of rate in different individuals. *Colias hyale* showed 54–66 forward pulsations per minute in the full-grown caterpillar but only 16 in antiperistalsis. In full-grown caterpillars of *Pararge mæra* he counted: 40 pulsations per minute at 20° C., 80 pulsations per minute at 30° C., 130 pulsations per minute at 40°C., but in a caterpillar suspended for pupation the number of pulsations fell from 40 to 25 at 20° C. He apparently did not observe reversal in this species until after pupation, when antiperistalsis appeared at the slow rate of 18 beats per minute or less. He argues that, if the heart is closed at the posterior end and provided with lateral valves by which a backflow of the blood would be made impossible, the facts which he has observed afford a physiological puzzle, for if the blood were driven back it would find at the blind end no means of exit.

He suggests that this puzzle may be solved by assuming that reversal of the blood stream is only an illusion. If the blood, as

is commonly believed, cannot flow backwards, it must during antiperistalsis still be flowing forward. It is interesting to note that Hecht ('18) had a similar view in regard to the reversal of peristalsis in the tunicate *Ascidia atra*, viz., that the flow is constantly advisceral in spite of the alternating advisceral ↔ abvisceral peristalsis.

Fischer sought an explanation of periodic reversal and the slowing of the pulse in antiperistalsis in the profound transformation of the structure of the body and especially in the change in the blood at pupation from an alkaline to an acid condition. The deep, slow, breathing which occur in human beings when acids accumulate in the blood, as in diabetes, he suggests, is somewhat comparable to the slow beating of antiperistalsis in insects.

The present writer in October 1924, while examining freshly formed chrysalids of *Colias eurytheme*, the alfalfa butterfly, quite unexpectedly and without any previous knowledge of the subject, observed antiperistalsis and periodic reversal. The following is a brief abstract of the report of these observations which appeared in *Science* and of a paper read before the American Society of Zoölogists (Gerould, 1924*a*, '24*b*).

The heart beats forward in the caterpillar until the approach of pupation. Then short backward phases alternate with longer forward phases. During pupation a long phase of double action, forward from the third, backward from the fourth, abdominal segment alternates with forward peristalsis. A few hours later, the double action becomes limited to a few (25) seconds followed by complete reversal. Except during pupation, when the phase of double action is inordinately long, the proportion between the length of the backward and the forward phases increases with age up to about 48 hours. Thereafter a decrease in the relative length of the backward phase and a slackening in rate occur. In the oldest chrysalis with visible pulse a long forward phase alternated, after a complete rest of several minutes, with a very brief backward action. The rate of beating backward is regularly about half that of beating forward, though the proportion of backward beats to forward changes and much individual variation occurs in the number of beats in a phase.

Reversal of heart-beat is an important feature of metamorphosis

connected with the rapid development of the wing buds and constriction of the base of the abdomen. Increased blood pressure in head and thorax thus relieves itself, either by complete direct reversal of peristalsis or by intermittent expulsion of hæmolymph backward through the thoracic sinuses into the base of the abdomen and up into the pericardium at the 3–4 abdominal segments, resulting in double action.

The constricted waist of holometabolous insects assists in the rapid increase in blood pressure necessary for the expansion of the wings. Such a constriction may serve a similar function in the rapid expulsion from the silk glands of large quantities of soft silk.

Search of the literature on circulation in insects gradually brought to light Malpighi's discovery of periodic reversal in the pupa and moth of the silkworm, Bataillon's excellent paper on metamorphosis in *Bombyx*, and the other literature already noted.

No observer since Malpighi, so far as I can ascertain, has given any definite information as to the real nature of the pulse in the adult moth or butterfly. Réaumur thought that pulsation in the adult was always backward; Cornalia believed that Malpighi was probably correct, but made no observations himself; Bataillon regarded periodic reversal as a transitory phenomenon connected with metamorphosis. Brocher ('17*a*, '17*b*, '19) who has made extensive and valuable studies on circulation in insects had apparently overlooked periodic reversal.

Such was the conflicting state of the case when I began in 1927 to study the silkworm. It did not take long to find that Malpighi was in the main correct as to periodic reversal in the adult moth. In the season of 1928 I confirmed the observations of the previous year on all stages from the prepupa onward and brought to light facts in regard to variations in the rate of backward and forward peristalsis which are to me of great interest. Some of these results were reported at the 4th International Congress of Entomologists, at Ithaca in August, 1928, and will appear in the proceedings of that session. A more complete account will be published in the *Journal of Morphology and Physiology*.

Only a single reference has yet been found to the occurrence of

this phenomenon in any other order of insects than Lepidoptera, viz., an interesting passage in Kirby and Spence (1828[6]) which suggests that in the adult drone fly (*Syrphus pyrastri*) periodic reversal may have been seen just a century ago. Observing the dorsal vessel through the transparent skin at the base of the abdomen, "which exactly forms such a window as physicians have sometimes wished for in order to view the interior of their patients," they remark:

"The included fluid does not run in the dorsal vessel in a regular course, but is propelled at intervals by drops, as if from a syringe, first from the wide end toward the trunk [thorax] and then in the contrary direction, forming a very interesting and agreeable spectacle."

LITERATURE CITED.

Bataillon, E.

1893 La métamorphose du ver à soie et le déterminisme évolutif. Bull. Sci. France et Belgique, 25, p. 18–55.

Brocher, F.

1917*a* Étude expérimentale sur le functionnement du vaisseau dorsal et sur la. circulation du sang chez les insectes. I[e] Partie. Le Dytiscus marginalis, Arch. de Zool. expér. et gén., 56, p. 347–358.

1917*b* II[e] Partie. Les larves des Odonates. Ibid., 56, p. 445–490.

1919 Les organes pulsatiles méso et métatergaux des Lépidoptères. Ibid., 58 p. 149–171.

Cornalia

1856 Monographia del Bombyce del Gelso. Mem. dell'I. R. Instituto Lombardo. Milano, 6, p. 388, 15 pls.

Fischer, E.

1918 Eine bei Raupen und Puppen beobachtete Umkehrung der peristaltischen Herzbewegung. Entom. Rundschau. Stuttgart. 35, p. 9–10.

Gerould, J. H.

1924*a* Periodic reversal of heart-beat in a chrysalis. Science, 60, p. 570–572.

1924*b* Periodic reversal of heart-beat in the chrysalis of Colias. Anat. Record, 29, 2, p. 90.

Hecht, S.

1918 The physiology of Ascidia atra, Lesueur. III. The blood system. American Jour. of Physiol., 45, p. 157–187.

Herold.

1823 Physiologische Untersuchungen über das Ruckengefäss der Insecten. Marburg, 67 pp.

Kirby, W., and W. Spence.

1828 An introduction to entomology. 4 vols. (*Vide* Vol. 4, p. 97.) London.

Maestri, A.

1856 Frammenti anatomici, fisiologici sul baco da seta. Pavia.

[6] Vol. 4, p. 97.

Maillot, E.

1878 Traité du ver à soie par Malpighi. Texte original et planches, avec une traduction et des notes en français. Station Séricicole de Montpellier. 12 pls., pp. 154.

Malpighi, M.

1669 Dissertatio epistolica de bombyce. London.

Réaumur, R. A. F. de.

1734 Mémoires pour servir a l'histoire des insectes. 1, p. 643. Paris, 1734–1742.

Verson, E.

1908 Sul vaso pulsante della sericaria. Atti del reale Inst. Veneto di sci., lett. ed arti., 67, p. 1291–1321, 2 pls.

Vieil, P.

1920 Sériciculture. Encyclopédie Agricole. Baillière et Fils. Paris.

FURTHER STUDIES ON THE SEX RATIO IN THE CHICKEN.[1]

W. V. LAMBERT AND V. CURTIS.

In the chicken, as in other animals, various attempts have been made to influence the proportion of the sexes normally obtained. While many claims have been made to the effect that it is possible to control sex, at least to a certain degree, most of these claims have not been substantiated when put to a careful analysis. Since such claims have been and will, no doubt, continue to be made it is desirable to have at hand a large body of data on the normal sex ratio for the fowl collected in different localities and over a considerable period of time.

It was in the hope that the observations here reported will add materially to this question, as well as to the question of the normal embryonic sex ratio in the fowl, that the present data were collected. Since the observations were made incidental to another problem, it is deemed wise to publish them at this time.

As these data were collected over a period of fourteen weeks and upon the chicks from hens of different ages they offer information as to the normal variability that may be expected in the sex ratio from week to week, and, also, from females of various ages. In addition, some data relative to the influence of previous and concurrent egg production on the sex ratio were accumulated.

Material and Methods.

The data reported herein were obtained primarily on the chicks from White Leghorn or White Leghorn by Rhode Island Red matings. In addition a few chicks were from pure Rhode Island Red stock. As a part of the chicks were obtained from sources other than pedigreed matings no attempt was made to keep the sex ratio for the two breeds and the hybrids separately.

All eggs were candled on the fourteenth and eighteenth days of

[1] Paper No. 30—Department of Genetics, Iowa State College, Ames, Iowa.

incubation and the sex of all embryos dead after the fourteenth day of incubation was determined by dissection. Likewise, the sex of all chicks dead before their sex could be ascertained from external examination, was made in a similar manner.

The sex ratio, as used in this report, expresses the percentage of males to females in the population.

The Normal Sex Ratio in the Chicken.

Most of the investigators who have reported upon the sex ratio of the chicken have observed a slight excess of females. The results of all the separate investigators with a total of all the results are shown in Table 1. A total of 38,907 observations have been

Table I.

A Summary of the Results of all Investigators of the Sex Ratio in the Chicken.

Author.	Year.	Total No. of Observations.	Ratio of ♂♂.
Darwin	1871	1,001	48.65±1.06
Field	1901	2,105	44.63±0.73
Thomson	1911	805	47.82±1.19
Pearl[1]	1917	22,791	49.45±0.22
Crew and Huxley	1923	753	49.26±1.23
Jull	1924	2,396	48.88±1.69
Mussehl	1924	1,514	52.24±0.87
Lambert and Knox	1926	2,910	51.13±0.62
Horn	1927	2,131	51.62±0.73
Lambert and Curtis	(This report)	2,501	46.82±0.67
Totals		38,907	48.76±0.17

[1] All sized families.

made, upon chicks and embryos, and the sex ratio for this total group is 48.76 ± 0.17. The lowest sex ratio, 44.63, was reported by Field (1901) and the highest, 52.25, by Mussehl (1924). The largest series of observations, 22,791, on the sex ratio in the chicken has been made by Pearl (1917). These observations were made over a period of eight years (1908–1915), and the range in the percentage of males reported by Pearl for the different years is from 46.16 to 49.99. Two other ratios above fifty have been reported, 51.13 (Lambert and Knox, 1926) and 51.62 (Horn, 1927).

The observations reported herein were made over a period of fourteen weeks, from April 11 to July 17, 1928. During this period a total of 2,501 chicks and embryos were examined for their sex. The total results listed by weeks for both chicks and dead embryos are given in Table 2. Of the 2,501 chicks and embryos examined 1,171 were males and 1,330 were females, or a sex ratio of 46.82 ± 0.67. Considerable variation was exhibited in the sex ratio for the various weeks, this ranging from 38.67 for the week of June 27 to 55.75 for the week of July 5. These are rather extreme deviations for the normal sex ratio, but as they are based upon rather small populations they are probably due entirely to chance. It is noteworthy, however, that in only three out of the fourteen weeks were sex ratios as high as fifty observed.

TABLE II.

THE SEX RATIO LISTED BY WEEKS THROUGHOUT THE HATCHING SEASON FOR ALL CHICKS AND EMBRYOS EXAMINED.

Date of Hatch.	Dead Embryos.		R. ♂♂.	Chicks.		Total.		R. ♂♂ Total.
	♂♂.	♀♀.		♂♂.	♀♀.	♂♂.	♀♀.	
April 11.....	34	38	47.22	57	63	91	101	47.39±2.43
" 18.....	67	68	49.62	3	2	70	70	50.00±2.85
" 25.....	55	64	46.21	13	17	68	81	45.63±2.76
May 2.....	12	20	37.50	57	67	69	87	44.23±2.70
" 9.....	37	43	46.25	46	42	83	85	49.40±2.60
" 16.....	21	27	43.75	77	79	98	106	48.03±2.36
" 23.....	13	13	50.00	115	133	128	146	46.71±2.04
" 30.....	10	5	66.67	66	85	76	90	45.78±2.62
June 6.....	15	14	51.72	47	78	62	92	40.25±2.72
" 13.....	15	23	39.47	72	81	87	104	45.54±2.44
" 20.....	13	4	76.47	102	104	115	108	51.56±2.26
" 27.....	11	13	45.83	30	52	41	65	38.67±3.27
July 5.....	6	5	54.54	62	49	68	54	55.73±3.05
" 17.....	15	23	39.47	100	118	115	141	44.92±2.11
Totals.....	324	360	47.36±1.29	847	970	1,171	1,330	46.82±0.67

No definite trend in the sex ratio is apparent as the season advanced, as both the lowest and the highest ratios appear in the last three weeks of the hatching season.

During the hatching season a total of 3,907 eggs were set and of this total 2,965 proved to be fertile. Of the fertile eggs, as determined by candling on the fourteenth day of incubation, 424

were embryos dead before the fourteenth day. A total of 684 embryos died between the fourteenth and twenty-first days of incubation, while 1817 of the eggs hatched. Forty of the chicks were lost before their sex was determined. These data, therefore, represent 84.35 per cent. of all the fertile eggs that were set. This figure is probably slightly high as some of the eggs classed as infertile must have been dead germs, although the percentage of such eggs cannot have been large.

Most of the chicks and embryos examined for their sex were from pedigreed matings, and the results for each colony of matings are listed in Table 3. Only one male was used in each of the

TABLE III.

THE SEX RATIO LISTED BY COLONIES. ONE MALE WAS USED IN EACH COLONY.[1]

Colony No.	No. of Females.	Dead Embryos.		Chicks.		Total.		R. ♂♂.
		♂♂.	♀♀.	♂♂.	♀♀.	♂♂.	♀♀.	
1.....	23	62	58	90	75	152	133	53.33±2.00
2.....	10	25	28	71	80	96	108	47.05±2.36
3.....	12	4	7	41	43	45	50	47.37±3.46
4.....	8	25	28	66	62	91	90	50.27±2.51
5.....	7	3	3	19	18	22	21	51.16±5.14
6.....	12	37	38	79	85	116	123	48.53±2.14
7.....	11	24	40	90	113	114	153	42.70±2.06
8.....	12	39	40	86	87	125	127	49.60±2.12
9.....	11	17	17	56	71	73	88	45.34±2.66
10.....	11	22	27	50	43	72	70	50.70±2.83
11.....	10	10	9	14	21	24	30	44.44±4.59
Totals ...		268	295	662	698	930	993	48.36±0.77

colonies. From this series of matings a total of 1,923 chicks and embryos was examined. Of this number 930 were males and 993 were females, the sex ratio being 48.36 ± 0.77. The range noted in the sex ratio of the different matings was from 42.70 to 53.33. While these are rather wide deviations from the average they are undoubtedly due to chance. When the sex ratio for each

[1] Some females were transferred from one colony to another during the course of the hatching season, after being away from the male of the first colony for a period of at least ten days. Altogether 79 females were used in this series of matings.

separate mating is considered it is found that most of the deviation in colony 1 is due to the aberrant ratio of two females and in colony 7 to the high percentage of females produced by three hens. In neither colony is the deviation great enough for any one hen, when both embryos and chicks are considered, to lead to the suspicion of factors other than chance having been responsible for the ratio in question.

The females used in this study were of different ages and the sex ratio from different aged females has been listed in Table 4. Five females were in their third or fourth year of production, 11 in their second year and 63 in their first year. The sex ratios for these three groups were 50.38, 44.03 and 48.79 respectively. While the number of birds in the first two groups is obviously too small to make sweeping conclusions it is apparent that age does not seem to modify the sex ratio greatly. The ratio of males is rather low for the two-year-old hens, but if reference is made to Tables 1, 2 and 3 it will be seen that deviations equally as great are not infrequent in populations as large or larger.

TABLE IV.

THE SEX RATIO CONSIDERED BY AGE OF DAM.

Year of Production.	No. of Females.	Dead Embryos.		Chicks.		Total.		R. ♂♂.
		♂♂.	♀♀.	♂♂.	♀♀.	♂♂.	♀♀.	
4	5[1]	18	21	48	44	66	65	50.38±2.62
2	11	21	30	75	92	96	122	44.03±2.28
1	63	229	244	539	562	768	806	48.79±0.85
		268	295	662	698	930	993	48.36±0.77

THE EMBRYONIC SEX RATIO IN THE CHICKEN.

In some species of animals, notably man, a considerable body of evidence has been presented to show that the primary sex ratio differs rather markedly from the secondary ratio. In fowls this does not seem to be the case. While the data on this question are not as extensive as might be desired they all point to the above conclusion. Pearl (1917) reports a sex ratio of 48.30 from a

[1] Four of the females were in their fourth year of production, one in the third.

total of 1,921 embryos examined from the tenth to twenty-first days of incubation. Jull (1924) a ratio of 42.10 in embryos dying naturally after the eleventh day of incubation, Thomson (1911) a ratio of 47.82 in 805 embryos, and Crew and Huxley a ratio of 45.24 in a total of 420 observations. Lambert and Knox (1926) observed a sex ratio of 51.43 in 1,048 embryos dead after the twelfth day of incubation, and Horn (1927) a ratio of 52.17 in 1,248 embryos examined from the tenth to the twenty-first days of incubation. This is a total sex ratio for dead embryos examined by all investigators heretofore of 48.80 ± 0.44.

In this study a total of 684 embryos dead before hatching were sexed. Of this group 324 were males and 360 females, giving a sex ratio of 47.36 ± 1.29. This result agrees well with the findings of other investigators, and when compared with the sex ratio of chicks it does not offer any evidence for a selective prenatal mortality of one sex in the chicken, at least during the latter part of the incubation period.

All of the observations on the embryonic sex ratio have been made only during the latter half, or less, of the incubation period. To change the sex ratio to an equality of males and females, it would be necessary to assume a very heavy mortality of males during the first half of the incubation period, and there is no good reason for believing that the early embryonic death ratio would be greatly different from that observed in the late stages of hatching.

Antecedent Production and the Sex Ratio.

Jull (1924) in a study of the sex ratio based upon continuous hatches throughout the year found a correlation of $-.704 \pm 0.031$ between the sex ratio and antecedent egg production. The ratio of males was found to decrease as the season advanced and total egg production increased, and Jull concluded that the cause for this decrease was directly related to antecedent egg production. Such a decrease was not noted by Jull during the normal hatching season.

Lambert and Knox (1926) did not find any significant correlation with the sex ratio, either for rate of production preceding the normal hatching season, or the actual production during the

normal hatching season. The respective correlations reported by them were $-.048 \pm 0.111$ and $-.009 \pm 0.108$.

Similar studies have been made for the data here reported. Correlations were calculated between the sex ratio and the rate of production for the three months preceding the hatching season, and for the actual production from March 1 to June 1. Only hens producing at least ten sexed offspring have been used in these calculations. The results with the sex ratio as the dependent variables are as follows:

Variables.	Correlation Coefficient.
A. Rate of production preceding the hatching season (per cent.)	$-.05 \pm 0.19$
B. Actual production (March 1 to June 1)..	$.09 \pm 0.13$

The size of neither of the correlation coefficients is great enough to indicate that there was a relationship between production and the sex ratio. While the number of hens upon which these observations were made was not large it is certain that the immediate antecedent production or concurrent production did not exert any noticeable influence upon the sex ratio of the chicks from these hens. These findings are in accord with those of previous investigators.

Summary.

1. The sex ratio for a total of 2,501 chicks and dead embryos examined from April 11 to July 17, 1928, was 46.82 ± 0.67. This represents the sex ratio upon 84.35 per cent. of all fertile eggs set during this period.

2. The sex ratio observed for dead embryos alone was 47.36 ± 1.29 and for chicks alone it was 46.61 ± 0.79.

3. Evidence is presented to show that there is not a selective mortality against one sex previous to the time of hatching.

4. No definite tendency of the sex ratio to increase or decrease was observed during the hatching season.

5. Separate tabulations for the sex ratio upon eleven colony matings, one male with several females in a colony, did not show significant differences between the colonies that might be traceable to individual differences.

6. No significant differences for the sex ratio of hens of different ages were noted.

7. Egg production for the three months immediately preceding the hatching season, or egg production during the hatching season did not influence the sex ratio. The respective correlation coefficients were $-.05 \pm 0.19$ and $.09 \pm 0.13$.

LITERATURE CITED.

Crew, F. A. E., and Huxley, J. S.

'23 The Relation of Internal Secretion to Reproduction and Growth in the Domestic Fowl. I. Effect of Thyroid Feeding on Growth Rate, Feathering, and Egg Production. Vet. Jour., 79: 343–348.

Darwin, Charles.

'71 The Descent of Man. Vol. 1, p. 296. D. Appleton and Co., New York.

Field, G. W.

'01 Experiments on Modifying the Normal Proportion of the Sexes in the Domestic Fowl. BIOL. BULL., 2: 360–361.

Horn, E.

'27 Untersuchungen über die Möglichkeit einer Geschlechtsvorausbestimmung beim Hühnerei nebst einer Factorenanalyse der Befiederungsgeschwindigkeit von Kücken. Zeitschr. Tierzucht. u. Zuchtungsbiol. 10: 179–224.

Jull, M. A.

'24 The Relation of Antecedent Egg Production to the Sex Ratio of the Domestic Fowl. Jour. Agr. Res., 28: 199–224.

Lambert, W. V., and Knox, C. W.

'26 Genetic Studies in Poultry. I. The Sex Ratio in the Domestic Fowl. BIOL. BULL., 51: 225–236.

Mussehl, F. E.

'24 Sex Ratios in Poultry. Poult. Sci., 3: 72–73.

Pearl, R.

'17 The Sex Ratio in the Domestic Fowl. Proc. Amer. Phil. Soc., 56: 416–436.

Thomsen, E.

'11 Die Differenzierung des Geschlechts und das Verhältnis der Geschlechter beim Hünchen. Arch. Entwick. Organismen, 31: 512–530.